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CALIFORNIA

COLLECTED REPRINTS OF OTTO CHARLES GLASER

1904-1925

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Away

PAMPHLET COLLECTION

Über den Kannibalismus bei Fasciolaria tulipa (var. distans) und deren larvale Excretionsorgane

(Biology)

DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

OTTO CHARLES GLASER

1904

UNIVERSITY

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Über den Kannibalismus bei Fasciolaria tulipa (var. distans) und deren larvale Excretionsorgane.

Von

O. C. Glaser
(Baltimore).

(From the Biol. Laborat. of the Johns Hopkins University Baltimore Md. U. S. A.)

Mit Tafel VI—IX und 5 Figuren im Text.

g.

Einleitung.

Fasciolaria tulipa gehört wegen ihrer höchst ungewöhnlichen Entwicklungsgeschichte zu den interessantesten Prosobranchiern, die an der südlichen Küste Nordamerikas vorkommen.

Abgesehen von den außerordentlich graziösen Eierkapseln, die sofort die Aufmerksamkeit fesseln, befindet man sich, sobald man ihren Inhalt näher betrachtet, vor einer ganzen Anzahl Probleme, von denen einige im Anfang jedenfalls etwas mysteriöser Natur sind. *Fasciolaria* weist nämlich einen im höchsten Grade entwickelten Kannibalismus auf, der, wie zu erwarten, mit andern Modifikationen verbunden ist. Es ist die Aufgabe dieses Beitrages über diese Verhältnisse Näheres zu berichten.

Mein Material wurde während dreier Sommer in Beaufort, North Carolina, gesammelt. Dort wurden mir durch die generöse Teilnahme der United States Fish Commission alle die nötigen Mittel zur Verfügung gestellt. Ich möchte daher meinem Danke für dieses liberale Entgegenkommen öffentlichen Ausdruck geben. Hauptsächlich bin ich dem Direktor des Beaufort Laboratoriums, Herrn Dr. CASWELL GRAVE, tief verbunden.

Das nähere Studium des Materials wurde im Biologischen Laboratorium der Johns Hopkins Universität, unter der Direktion meines hochverehrten Lehrers, Herrn Professor Dr. W. K. BROOKS, ausgeführt. Ihm sowohl als seinen Assistenten, und meinen Freunden im Laboratorium, spreche ich meinen herzlichsten Dank aus.

Meinem Vater verdanke ich den chemischen Teil dieser Arbeit und teilweise die Bereitung des Manuskriptes.

Technik.

Von allen den Fixierungsmitteln, mit denen ich arbeitete, gab KLEINENBERGS Pikrin-Schwefelsäure die besten Resultate, für Schnittserien sowohl als für ganze Präparate. Färbung wurde mit Boraxkarmin, MAYERS Hämalaun, KLEINENBERGS Hämatoxylin und CONKLINS Modifikation von DELAFIELDS Hämatoxylin vollzogen. Diese Färbmittel gaben annähernd gleich gute Resultate, doch für eingehendere Beobachtungen war Boraxkarmin am besten. Dünnschnitte wurden mit den genannten Färbmitteln behandelt, aber auch mit HEIDENHAINS Eisenhämatoxylin. Gegen mein Erwarten gaben Boraxkarmin und MAYERS Hämalaun einige meiner besten Präparate. Doppelfärbungen wurden mit Bleu de Lyon und Eosin gemacht.

Das Anfertigen von Dünnschnitten gab Schwierigkeiten, die zum Teil noch nicht überwunden sind. Wie bekannt, wird Dotter durch Dehydration außerordentlich spröde. Bei *Fasciolaria* wird ein einzelnes Ei durch gewöhnliche Behandlung dermaßen hart, daß es imstande ist, die Messerschneide, wo sie auch getroffen wird, sofort zu verderben. Meine besten Resultate wurden dadurch erzielt, daß ich die höheren Alkohole und Xylol ganz umging, wenn ich in Paraffin einbetten wollte. Dies wurde dadurch erzielt, daß ich sofort von 70, 75 oder 80% Alkohol zu Kreosot überging. In diesem Reagenz wurden die Larven klarer, aber, wie ich glaube, nicht gänzlich dehydriert. Vom Kreosot ging ich unmittelbar zu Paraffin über, in welchem, nach halbstündiger Immersion eingebettet wurde. Nach dieser Behandlung wurden die Larven fast nie spröde, und in manchen Fällen gelang es mir, Dünnschnittserien durch Massen von über 300 Eiern anzufertigen. Unter dem Mikroskop zeigte es sich, vor Auflösung des Paraffins, daß die Imprägnation eine unvollkommene war, jedoch genügend, um die Objekte an Stelle zu halten. Obgleich ich dieser Methode keine allgemeine Anwendbarkeit zuschreiben möchte, ergab sich doch, daß sie befriedigende Schnitte durch die Eier eines Fisches (*Batrachus tau*) lieferte. Änderungen der gewöhnlichen Färbungstechnik wurden durch den Gebrauch von Kreosot nicht nötig.

Eiablage.

Die Eiablage von *Fasciolaria* findet in Beaufort in den Monaten Mai und Juni statt. Mitte Mai werden Eier in allen Stadien der

Entwicklung gefunden, aber Ende Juni findet man nur die späteren Stadien und Mitte Juli sind die meisten Kapseln leer, obgleich man immer noch vereinzelte Kapselgruppen in sehr verschiedenen Stadien selbst im August noch finden kann. Die Eikapseln werden in Gruppen auf Austerschalen, entweder über der Flutlinie oder gerade unter der Ebbelinie abgelegt. In ihrem frischen Zustande sind sie auffallende, durchsichtige Objekte, die durch ihren Inhalt entweder rötlich oder weiß gefärbt sind. Anfangs weich, werden sie nach längerem Aufenthalt im Wasser sehr elastisch und verlieren nach und nach ihre Schönheit dadurch, daß braune oder grüne Algen und andre Gewächse sich an ihnen befestigen.

Die Größe dieser Gruppen ist verschieden, da die Anzahl der Kapseln sehr wechselt. Im großen und ganzen haben die Gruppen einen Längsdurchmesser von 2—7 cm, jedoch kommen auch vereinzelte Kapseln vor, die einen kurzen Durchmesser von 0,5 cm, einen langen von 1,5 und eine Höhe von 1,5—2 cm aufweisen.

Vollauf so gewöhnlich wie vereinzelte Kapseln, kommen Gruppen von 30—35 vor; jedoch die Mehrzahl weist ungefähr 20 Kapseln auf. Diese Zahl, die zum Teil davon abhängt, ob, oder wie oft, das Weibchen während der Eiablage gestört wurde, wird auch durch die Größe und das Alter des Weibchens beeinflußt. Die älteren und größeren Weibchen deponieren nicht nur die größten Gruppen, sondern auch die größten Kapseln.

Die Eier, die anfangs ganz unregelmäßig im eiweißartigen Inhalte der Kapseln suspendiert sind, sind weiß, rötlich oder bräunlich gefärbte Sphären von 0,17—0,25 mm Durchmesser, mit einem klaren, animalen Pole, in dem sich ein großes, excentrisch gelegenes Keimbläschen befindet. Die Zahl dieser Eier ist ganz erstaunlich groß. Durch wirkliches Zählen des Inhalts einer Kapsel gelangte ich zu der Zahl 2308. Die höchste Abschätzung, der ich mich aus der Literatur erinnere, ist von 600—800. Wer Erfahrung mit den Täuschungen, die eine Anzahl Eier in einem Uhrgläschen hervorrufen kann, gemacht hat, kann kein Vertrauen mehr zu Abschätzungen haben.

Von diesen Eiern ist aber nur eine ganz kleine Anzahl befruchtet. Beinahe alle bleiben länger im Stadium liegen, in dem sie abgelegt wurden, sogar das Keimbläschen bleibt während 4—5 Tagen ganz unverändert. Mittlerweile hat sich die kleine Anzahl befruchteter Eier zu Larven entwickelt, die jetzt durch heftige Cilienbewegungen sich selbst, sowohl als die in dem Kapselschleim verteilten unbefruchteten Eier zu einem dichten Haufen in der Mitte zusammentreiben. Dort werden die Eier dann von den Embryonen in ganz unglaublicher Quantität verzehrt. Eine mittelmäßig große Larve, die ich öffnete, und deren Inhalt ich zählte, enthielt über 300 Eier; viele enthalten zweifellos mehr.

Wegen der Undurchsichtigkeit aller Eier und der Seltenheit der befruchteten, ist es mir nicht möglich gewesen, die letzteren direkt zu zählen. Ich konnte mir aber eine Idee durch die Anzahl der Larven, die durchschnittlich in den Kapseln vorkommen, machen. Die Anzahl der befruchteten Eier kann nicht kleiner sein als die Anzahl der Larven der Kapseln. Es sind vielmehr Gründe, wie das Vorkommen von Zwerglarven, die leicht übersehen werden, und die vielen Unfälle, die in den früheren Stadien häufig vorkommen, auf die ich später noch zurückkommen werde, zur Annahme vorhanden, daß die erstere Zahl größer ist, als die zweite. Ich konnte daher nur einen annähernden Schluß ziehen, der auf 145 Kapseln basiert ist, in denen durchschnittlich 6,2 Larven vorkamen. Die Extreme waren 15 und 2, zwischen denen die andern Zahlen vorkamen.

Die Entwicklungsstadien, die in einer Kapsel vorkommen, sind so verschieden, wie die Anzahl der Larven; z. B. findet man Zweizellstadien und ältere Larven durcheinander. Manche Kapseln zeigen schon direkt nach der Ablage zweizellige Stadien, während andre diese nur nach mehreren Stunden aufweisen. Es ist daher nicht leicht zu bestimmen, wie schnell sich ein Ei entwickelt, besonders weil die Eier, wenn sie aus den Kapseln entfernt werden, sich nicht normal weiter teilen. Ich machte meine Beobachtungen daher meistens, indem ich die Zeit der Ablage genau feststellte, nach kürzerer oder längerer Zeit die Kapseln öffnete, und mit dem Mikroskop die Stadien heraussuchte. Man kann im embryonalen Leben der jungen *Fasciolaria* folgende Stadien unterscheiden: 1) Die Pre-Kannibalperiode, 2) die Kannibalperiode, 3) die Veligerperiode, 4) die Periode der jungen Ausgewachsenen. Folgende Tabelle gibt das Resultat aller meiner Beobachtungen über die Dauer der Entwicklungsperiode.

Pre-Kannibalperiode	Kannibalperiode	Veligerperiode	Periode der jungen Ausgewachsenen
Bis zum 3. oder 4. Tage nach der Ablage.	Vom 4. bis zum 7. Tage nach der Ablage.	Vom 7. bis zum 20. Tage nach der Ablage.	Vom 20. bis zum 40. Tage nach der Ablage.

Pre-Kannibalperiode.

Die Schwierigkeiten, die sich dem Studium der früheren Stadien des *Fasciolaria*-Embryos entgegensetzten, sind der Art, daß es mir, sowenig wie andern, gelang, eine genügende Zahl Präparate anzufertigen, um genauer in den Furchungsprozeß eingehen zu können. Ich will aber anführen, daß, soweit wie ich beurteilen kann, nichts in den ersteren Stadien vorkommt, was nicht schon an viel günstigerem Material beschrieben worden ist.

Am Schlusse des Furchungsprozesses, nachdem der Blastoporus sich geschlossen hat, entsteht eine Larve, die in Fig. 1 wiedergegeben ist. Central gelegen ist eine Masse Dotter, die, wie spätere Stadien genau zeigen, den Rest der vier Makromeren repräsentiert. Um diese Dottermasse herum ist eine außerordentlich dünne Membran, die nur an dem vorderen Ende, wo sie etwas von dem Dotter absteht, genauer sichtbar ist.

Dort kann man Näheres von ihrer Struktur sehen. Zellwände sind zu dieser Zeit nicht sichtbar, obgleich sie in früheren Stadien klar erscheinen. Die Kerne des Ectoderm sind sehr granulär, und jeder ist von einer Anzahl Vacuolen, die nach außen zu immer kleiner werden und zuletzt verschwinden, umgeben. Außerhalb der kleinsten Vacuolen sind hier und da Körnchen, die die Zellen wenig bestimmt begrenzen.

Die weitere Entwicklung dieser Larve ist hauptsächlich durch die Vergrößerung des Hinterteiles der Ectodermal-Eihülle charakterisiert. Das Resultat ist die außergewöhnliche Form, die in Fig. 2 wiedergegeben ist. In diesem Stadium ist die Larve nur weniger komplex als im früheren.

Nach vorn projektiert sich vom Dotter eine dünne mit Vacuolen versehene Ectodermkappe. Am hinteren Ende hat sich die größte Veränderung vollzogen. Hier ist die dünne Ectodermalhülle, die mit der vorderen Kappe (*Kpf.bl*) in Verbindung steht, dermaßen gewachsen, daß sie sich jetzt nicht, wie früher, so glatt an den Dotter anlegt, daß sie überhaupt nur sehr schwer zu sehen ist, sondern sie erscheint viel zu groß für den Dotter (*hin.Bl*). Die Zellen dieser hinteren Kappe enthalten, im Gegensatz zu denen der vorderen, nicht so viele Vacuolen; auch ihre Zellwände sind sichtbar.

Auf Schnitten erweist es sich, daß wir es hier mit einem dünnen Säckchen zu tun haben, welches wahrscheinlich mit einer klaren, durchsichtigen Flüssigkeit gefüllt ist. Die Wände dieses Säckchens sind dünn, wie die der vorderen Blase (Fig. 4 *hin.Bl.—Kpf.bl*), scheinen aber weniger steif zu sein. Hierauf ist wahrscheinlich die höchst unregelmäßige Form, die nie in zwei Individuen desselben Alters gleich ist, zurückzuführen. Solche Formen finden ihresgleichen, so viel ich weiß, nur in den Beschreibungen von KOREN und DANIELSSEN (57). Ich selbst war längere Zeit der Ansicht, sie seien Abnormitäten, jedoch wurde ich durch ihr regelmäßiges Vorkommen in allen Kapseln des richtigen Alters, gezwungen, sie als ein typisches, normales Stadium anzusehen.

Die gefaltete irreguläre Oberfläche der Larve hat, selbst in einem früheren Stadium, als das in Fig. 2, eine konstant vorkommende Unregelmäßigkeit vor dem Mittelpunkte des langen Durchmessers. Diese Unregelmäßigkeit ist eine zylindrische Geschwulst, die senkrecht zur langen Achse steht (Fig. 2 *Cyl*). Von der Seite gesehen, erscheint dieser Zylinder wie ein hohler Zellring, aber durch vorsichtiges Einstellen des Mikroskops kann man die dünne Membran finden, die das obere und untere Ende schließt.

Ein Querschnitt durch eine Larve in diesem Alter ist in Fig. 3 wiedergegeben. In der Mitte zeigt sich der Dotter, anscheinbar in einer Masse, die durch ein dünnes Häutchen (*Ht*) — wahrscheinlich direkt vom Ei abstammend — umgeben ist. Dotterkügelchen von verschiedenen Größen liegen unregelmäßig verteilt im eingeschlossenen Raume, der peripherisch direkt unter dem Häutchen einen gekörnten Ring aufweist (*gr.Rg*).

Außerhalb des Häutchens ist eine entodermale Membran, die in diesem Falle aus drei äußerst gestreckten spindelförmigen Zellen besteht (*End*).

Gerade außerhalb des Entoderm ist das Mesoderm (*Mes*). Zu dieser Zeit fehlen dem Mesoderm definitive Zellwände, die Kerne aber sind deutlich charakterisiert in einem Substratum, welches sich nur wenig färbt. Rechts und links der Mittellinie sieht man Kerne, die in dem einen Falle in der Teilung begriffen sind, im andern sich schon geteilt haben. Dieser Teilungsprozeß scheint amitotisch zu sein, ich habe aber nicht genau feststellen können, was aus den Kernen wird.

Das Ectoderm, welches das einzige der Keimblätter ist, das jetzt schon den Dotter ganz umgibt, hat drei charakteristische Regionen. Unten sind die Zellen gleich dem Entoderm lang und spindelförmig; seitlich, im oberen Teile, den rechten und linken Enden des Zylinders entsprechend, sind zwei Gruppen von runden Zellen mit großen Kernen (*Ex.K*), welche die Anlagen für die bei *Fasciolaria* höchst wichtigen Organe der Larven — die Excretionskörper sind.

Die oberen Zellen dieser Außennieren haben Fortsätze, die mit gewöhnlichen spindelförmigen Zellen verbunden sind, welche die Oberfläche des Zylinders vervollkommnen.

Der Zylinder und ein Teil seiner Zellen spielen in der weiteren Entwicklung eine äußerst wichtige Rolle.

Fig. 5 zeigt einen Horizontalschnitt durch einen etwas älteren Zylinder. Der Hohlraum, der von den Wänden eingeschlossen ist, ist die Höhle des Zylinders. Die Vorderwand hat viereckige oder ovale Zellen. Seitlich sind die zwei Gruppen (*Ex.K*), die ein etwas fortgeschrittenes Stadium darstellen, in dem schon einige der sich später noch viel verstärkenden Vorgänge zeigen. Die Hinterwand des Zylinders besteht aus bewimpertem Ectoderm, dem Zellenwände fehlen. Anstatt dessen findet man hier ein Syncytium (*Syn*), in dem Kerne und Vacuolen unregelmäßig vorkommen.

Die Abwesenheit der Zellwände — das Vorkommen der Vacuolen, sind Zeichen der Degeneration. Daß diese Vorstellung die richtige ist, glaube ich deshalb, weil an diesem Orte der Mund durchbricht. Zu dieser Zeit also sind die definitiven Achsen der jungen *Fasciolaria* schon bestimmt.

Die Periode des Kannibalismus.

Nachdem die Mundöffnung entstanden ist, fängt sofort die Periode des Kannibalismus an. Die unbefruchteten Eier, die bis zu dieser Zeit unregelmäßig in dem eiweißartigen Inhalte der Kapseln verbreitet waren, werden jetzt durch eine aktive Wimperbewegung, die zu seiten der Mundöffnung der Embryonen stattfindet, zu einer kompakten Masse in der Mitte der Kapseln zusammengetrieben.

Einige Stunden nach dieser Konzentration der Eier kann man in dieser Masse kleinere, dichtere Gruppen wahrnehmen, und nach 2—4 Tagen sind alle Eier in von 2 bis 15 solcher Pakete enthalten. Diese Pakete, welche einen Durchmesser von 1,48 mm haben, sind die Kannibalen, welche sich ganz unglaublich gemästet haben.

Eine Serie solcher Masttiere habe ich in Fig. 6, 7, 8, 9, 10, 12 wiederzugeben versucht. In Fig. 6 ist eine Larve, auf ihrer linken Seite liegend, kurze Zeit nach Durchbruch des Mundes, abgebildet. Larven zu dieser Zeit sind bedeutend größer, als die schon beschriebenen, wahrscheinlich durch Aufnahme von Eiweißsubstanz, in welcher sie suspendiert sind. Rechts und links vom Munde sind die Excretkörper (*Ex.K*). Die vier Dotterkugeln, die nahe dem mittleren Teile des Rückens erscheinen, sind die Überbleibsel der vier Makromeren, die in den früheren Stadien so sehr zusammengedrückt waren, daß man sie nicht als separate Zellen erkennen konnte. Später lösen sie sich auf und werden als Nahrungsmaterial aufgebraucht.

In Fig. 7 zeigt sich in einer Larve desselben Alters, wie die in Fig. 6 wiedergegebene, der Anfang des Kannibalismus. Diese Larve hat zwei Eier verschluckt. Die vier Makromeren (*Mak*) sind von ihrer primären Stellung etwas verschoben, da nur zwei von ihnen unter dem rechten Excretkörper sichtbar sind. Diese Larve ist augenscheinlich von der erstbeschriebenen sehr verschieden, doch sind ihre Unterschiede weder auf Struktur noch Alter zurückzuführen, sondern erklären sich dadurch, daß, wie in den früheren, so auch in den jetzigen Stadien von *Fasciolaria*, überhaupt keine zwei Larven sich gleich sind.

Eine Larve, die vier Eier verschluckt hat, und die ein fünftes bewältigt, ist in Fig. 8 wiedergegeben. Mit Fig. 7 verglichen ist diese Larve von rechts gesehen. Sie unterscheidet sich von den bisher beschriebenen hauptsächlich durch das Verhalten ihres Kopfbläschens und der Excretkörper. Diese Eigentümlichkeiten sind auf das Alter der Larve zurückzuführen — denn obgleich sie schon mehr Eier verschluckt hat als die andern, ist sie dennoch in Wirklichkeit jünger. Das öftere Vorkommen von Fällen dieser Art führte anfangs zu Verwirrung. Ich lernte jedoch bald, daß die Anzahl Eier, die eine Larve verschluckt hat, überhaupt keine Vorstellung über ihr Alter geben kann, denn manche, die ich später besprechen werde, verschlucken gar keine. Darum ist in dieser Serie die Ordnung nicht auf Alter, sondern auf die Anzahl der verschluckten Eier basiert.

Fig. 9 zeigt die ventrale Seite einer Larve, die 14 Eier verschluckt hat. Dieser Embryo fängt schon an, die Regelmäßigkeit, die den späteren Stadien charakteristisch ist, zu zeigen. Ich glaube, daß diese auf mechanischen Kräften beruht — denn mit der Zunahme der verschluckten Eier wird die Larve von innen heraus geglättet und wird schließlich, wie zu erwarten, sphärisch (Fig. 10).

Dieses Bild (Fig. 10) ist für das Stadium der vollgestopften Kannibalen charakteristisch. Von der dünnen, durchsichtigen Körperwand ist beinahe nichts zu sehen, nur vorn kann man das etwas unregelmäßig gefaltete Kopfbläschen (*Kpf.bl*) wahrnehmen.

Ein Dünnschnitt durch eine solche Larve (Fig. 11) zeigt zwei

Membranen — Ectoderm und Entoderm (*Ect*, *End*). Sie bestehen zu dieser Zeit aus spindelförmigen Zellen, deren langausgedehnte Fortsätze miteinander in Verbindung stehen. In Schnitten durch andre Ebenen geführt, zeigt sich auch das Mesoderm, welches den ganzen Embryo noch nicht umwachsen hat. Da der Schnitt vor und durch einen Embryo geführt ist, der etwas älter war, als der in Fig. 10, so zeigen sich auch einige Veränderungen, die in den ingestierten Eiern vorgehen. Manche dieser Eier haben schon ihre Membranen verloren, und die Dotterkörnchen liegen lose im Darm. In andern, bei denen die Häutchen noch ganz sind, fangen die Keimbläschen, die eine Woche oder länger nach Ablage der Eier anscheinend unverändert bleiben, an sich in Stückchen zu teilen, deren jedes eine Miniatur des primären Keimbläschens ist, d. h. ein jedes hat einen großen Chromatinnucleolus, der von klarem Nucleoplasma umgeben ist. Diese Teilungen sind zweifellos mit der Senescenz der Eier verbunden.

Die Ventralseite eines älteren aus Schnittpräparaten wieder zusammengesetzten Kannibalen ist in Fig. 12 wiedergegeben. Kopfbläschen, Außennieren, Mund und Fuß sind einem Individuum entnommen, der übrige Körper einem andern. Das Kopfbläschen ist größer als wie gewöhnlich, doch ist es in andrer Hinsicht ganz normal. Das Bild zeigt genau die an einem einzelnen Exemplar überhaupt nicht wahrzunehmenden Verhältnisse der äußern Organe. Direkt unter dem Kopfbläschen befindet sich die Mundöffnung, der rechts und links die Excretkörper anliegen. Unter der Oralöffnung ist eine runde Ectodermalscheibe, die rechts und links zwei viertelmondförmige Verdickungen aufweist. Diese Scheibe ist die Anlage des Fußes (*Fs*), die scheinbar, wie schon von andern Autoren angegeben, auch bei *Fasciolaria* eine doppelte ist. Zwischen den Excretkörpern, dem Kopfbläschen, dem Munde und der Fußanlage ist das Velarfeld, aus dem sich später das Velum entwickeln wird.

Welches sind die regulativen Faktoren im Kannibalismus der Larven? Hat die Larve Eigenwillen oder ist sie Automat? Wie schon angeführt, findet man Eier in allen möglichen Stadien der Teilung in derselben Kapsel. Ebenso findet man in älteren Kapseln Larven von sehr verschiedenen Altersgraden. Von diesen werden die ältesten, d. h. die, die am frühesten bereit sind, die unbefruchteten Eier zu vertilgen, die meisten bekommen, während den sich minder schnell entwickelnden nur die Überbleibsel zufallen. Sehr spät zum richtigen Stadium gelangte bekommen oft gar keine, entwickeln sich jedoch eine Zeitlang in scheinbar normaler Weise als Zwerge weiter. Die meisten dieser Zwerge gehen zugrunde, jedoch werden sie öfters als verkümmerte Tiere aus den Kapseln entlassen.

Von den sich mit gleicher Geschwindigkeit entwickelnden Larven haben die mit den größten Oralöffnungen und der stärksten Wimperbewegung im Adoralfelde den Vorzug. Es folgt daraus, daß im großen und ganzen diejenigen, die am frühesten zur Mahlzeit kommen und am schnellsten und am leichtesten schlucken können, die meisten Eier in sich aufnehmen.

Zahlreiche Experimente wurden gemacht, um das Resultat einer künstlichen Vermehrung der verschluckten Eier festzustellen. Eines dieser Experimente hatte ein erstaunliches Ende.

Es ist mir nicht möglich gewesen, die Larven außerhalb der Kapseln auf längere Zeit normal weiter zu ziehen. Das Problem, die Nahrung einer gewissen Larve künstlich zu vergrößern, hatte daher seine Schwierigkeit, speziell, da es mir auch nicht gelang, eine einmal geöffnete Kapsel wieder auf befriedigende Weise zu schließen. Endlich kam mir der Gedanke, einige Larven in den Kapseln zu zerquetschen, und die von ihnen verschluckten Eier den unverletzten Larven zu offerieren. Dieses gelang mir denn auch, und mit Leichtigkeit, da eine kleine Verletzung einer Larve die Eier herausrollen ließ, wie Korn aus dem Loche eines Sackes. Auf diese Art und Weise wurden in manchen Kapseln Tausende von Eiern freigemacht. In meinen Experimenten wurden nach und nach alle bis auf zwei oder drei Larven verletzt, und ihr unverdauter Inhalt den Unverletzten überlassen. Diese, obgleich schon bis zum Platzen voll, machten sich in jedem Falle an die unerwartete zweite Mahlzeit und hatten in 3—4 Tagen das Doppelte ihrer normalen Quantität Eier verschluckt. Eines dieser Experimente wurde so weit geführt, daß schließlich nur noch ein einziger Embryo in der Kapsel übrig blieb. Diese Larve fuhr ruhig fort, die Eier, von denen jedes wenigstens schon einmal, und die meisten zwei- und dreimal verschluckt worden waren, zu vertilgen, aber die Elastizität der Körperwand hatte ihre Grenzen, und das Tier platzte schließlich durch Überfütterung. Überfütterung, wie sie hier stattfand, kommt auch in der Natur vor. Man kann sich leicht denken, daß die natürlichen Zerrungen und Störungen, denen die Kapseln im Wasser ausgesetzt sind, viele von den äußerst zarten Larven verletzen, und daß die auf diese Weise freigelegten Eier den andern zugute kommen. Daß dies auch wirklich der Fall ist, glaube ich daraus schließen zu dürfen, daß in den Kapseln, in welchen nur zwei oder drei Larven vorhanden sind, die Embryonen ausnahmslos größer sind, als im Durchschnitt.

Aus diesen Beobachtungen darf ich wohl folgern, daß eine Larve keine Kontrolle über den Kannibalismus hat, sondern daß die Anzahl der verschluckten Eier von der Anzahl der konkurrierenden Geschwister, und der Elastizität des eignen Körpers abhängt.

Die Entstehung des Kannibalismus.

Die Ingestion der Eier und Embryonen bei *Fasciolaria* ist den Fällen bei andern Gastropoden ähnlich, in welchen die Larven sich entweder gegenseitig verzehren, oder sich auflösen zugunsten der andern. Es schien mir gerechtfertigt, alle solche Fälle der Ernährung zusammen als Kannibalismus zu fassen. Ich halte die Entstehung dieses eigentümlichen Verfahrens nicht für so einfach, wie es aus manchen Auseinandersetzungen folgen sollte.

McMurrich (86) referierte darüber, daß die »ova« in den Kapseln von *Crepidula fornicata*, *C. plana* und *C. convexa* sich teilweise auflösen und von den gesunden Embryonen verschlungen werden. Dasselbe zeigte sich noch ausgesprochener bei *Purpura floridana*.

Koren und Danielssen (56) fanden in den Kapseln von *Buccinum undatum* einen ähnlichen Unterschied in der Anzahl der älteren Embryonen und der Eier. *Buccinum* unterscheidet sich von *Fasciolaria* dadurch, daß viele von den Eiern, die sich nicht zu Embryonen entwickeln, sich trotzdem furchen. Carpenter (57) korrigierte die Ansichten von Koren und Danielssen über die Entstehung der Embryonen durch Konglomeration, indem er die Entwicklung von *Purpura lapillus* beschrieb, in welcher von 500—600 Eiern sich 12—30 zu Embryonen entwickeln, während der Rest sich unregelmäßig in 14—20 Teile teilt.

Selenka (72), der Carpenters Resultate bestätigte, glaubte nicht, daß die Teilungen der »sterilen« Eier dem Furchungsprozesse der Befruchteten entspräche — nicht nur, weil der erstere Prozeß so unregelmäßig verläuft, sondern weil gar kein Kern vorhanden sein soll. In den Kapseln der *Neritina fluviatilis* sollen nach Blochmann (82) alle Eier mit Keimbläschen versehen sein, die sich in der gewöhnlichen Weise an der Bildung der Richtungskörper und des weiblichen Pronucleus beteiligen sollen. Nach diesen Vorgängen jedoch sollen die »sterilen« Eier sich so unregelmäßig betragen, daß Blochmann sich gezwungen fand mit Bütschli (77) in des letzteren Annahme — sie seien unbefruchtet, übereinzustimmen. Brooks (78) beobachtete, daß die 6—20 Eier in den Kapseln der *Urosalpinx cinerea* normalerweise sich alle entwickeln, daß aber zuweilen etliche derselben degenerieren und von den gesunden Larven als Nahrung gebraucht werden. Dieser Fall von ausnahmsweisem Kannibalismus gab McMurrich (loc. cit., p. 408) »A clue to the manner in which the phenomena seen in *Fasciolaria*, *Purpura lapillus* etc., have been brought about. An occasional egg in a capsule has from some cause or other broken down, and has been drawn into the digestive cavity of the developing embryos. This process having proved useful, is continued, and an arrangement such as I have described above for *Purpura floridana* obtained. From this it is but a step to what occurs in *Buccinum*, *Purpura lapillus* and *Neritina*. In *Fasciolaria* the process is, as far as we know at present, at its culmination.«

Abgesehen von den Schwierigkeiten, die sich in dieser einfachen Auffassung entgegensetzen, ist ein andrer Faktor, wie McMurrich selbst angibt, stets beim ausgesprochenen Kannibalismus vorhanden. »This is (loc. cit., p. 409) the non-fertilization of the majority of the ova, whereby it is impossible for them to develop to any great extent, and whereby they naturally break down when they have endeavoured to segment. We see this in *Neritina*, *Buccinum* and *Purpura lapillus*. In *Fasciolaria*, as stated above, the process reaches its climax, and in this case the sterile nutritive ova do not show the least trace of segmentation, nor do they ever show signs of maturation.«

Dieser zweite Faktor scheint mir von großer Wichtigkeit zu sein und wirft, da wir jetzt mehr über die Entwicklung der Geschlechtszellen bei den Gastropoden wissen, ein neues Licht auf die Entstehung des Kannibalismus.

Erstens mögen die eingeführten Stoffe, ungefurchte Eier, Teile gefurchter Eier, oder Embryonen, oder eine Mischung von irgendzweier oder dreier dieser Bestandteile sein. Bei *Neritina* und *Fasciolaria* z. B. werden ungefurchte Eier aufgenommen; bei *Buccinum* meistens gefurchte Eier, zuweilen ungefurchte; und bei *Crepidula*, *Purpura* und *Urosalpinx* Derivate von früheren oder späteren Entwicklungsstadien. Obgleich *Fasciolaria* in dieser Hinsicht neben *Neritina* steht, gleicht sie auch *Crepidula*, *Purpura* und *Urosalpinx*, denn die abnormen Larven der Pre-Kannibalperiode, sowohl als manche der Zwerglarven und andre Abnormitäten späterer Perioden, werden von den normalen Larven aufgebraucht. Die Entstehung des Kannibalismus reduziert sich demnach auf zwei Fragen: Wie soll man erklären, daß so viele Embryonen zugrunde gehen? Warum sind so viele Eier unbefruchtet?

Ich glaube nicht, daß die Aufnahme dieser Substanzen Erklärung braucht, denn sie sind ja zweifellos als Nahrung nützlich. Daß manche der Embryonen sich auflösen, kommt, in *Fasciolaria* wenigstens, daher, daß sie von den Geschwistern, die das Glück haben, von schnellerfurchenden Eiern abzustammen, weit im Hintergrunde gelassen werden. Andre Gründe liegen auch auf der Hand — z. B. die Größe der Eier — die Widerstandsfähigkeit der Larven gegen die Schädigung durch Aussetzung bei Ebbe — oder die Störungen und Verletzungen, denen die Kapseln normalerweise preisgegeben sind. Ich kann mir nicht denken, daß das Zugrundegehen der Embryonen auf vergangener Zuchtwahl dieser Eigenschaft beruhe.

Daß so viele Eier, wie bei *Fasciolaria*, *Neritina* (BÜTSCHLI, BLOCHMANN) und *Purpura* (SELENKA) unbefruchtet bleiben, ist nicht so leicht zu verstehen, obgleich sich in *Purpura*, in der »Abwesenheit des Keimbläschens«, ein Grund finden lassen mag. Die unbefruchteten Eier der *Fasciolaria* reagieren nicht auf Chemikalien, obgleich Herr Professor CONKLIN durch ein paar Tropfen Ammoniak kleine Schwellungen an ihnen erzeugen konnte. Künstliche Befruchtung gelang mir unter den verschiedensten Umständen nicht, und in keinem meiner Experimente schienen die Eier die Spermien auch nur im geringsten Grade anzuziehen. Da bei den Gastropoden die Befruchtung normalerweise vor der Bildung der Richtungskörper stattfindet, bin ich zu der Ansicht gelangt, daß die Eier unvollkommen sind.

Wie ist diese Unvollkommenheit zu erklären? Ich lehne mich hierbei an die höchst interessanten Resultate von MEVES (02). Bekanntlich hat MEVES über die Spermatogenese der oligopyrenen oder wurmähnlichen Spermien, und den eupyrenen oder haarähnlichen Spermien von *Paludina* Näheres berichtet. Unterschiede in der Entwicklung dieser zwei Arten zeigen sich erst im Stadium der primären Spermatocyten, dadurch, daß zwei Hauptgrößen vorkommen — die größeren liefern schließlich die oligopyrenen Spermien, die kleineren die eupyrenen Formen. Wenn die primären Spermatocyten reifen, findet in der größeren Art keine Reduktion statt — die normale Zahl der Chromosomen 14 erscheint in dem sich teilenden Kerne. Diese Chromosomen werden so in der folgenden Teilung verteilt, daß eine der Tochterzellen (sekundäre Spermatocyten) 10 bekommt, die andre nur 4. Wenn sich dann die sekundären Spermatocyten teilen, so teilt sich nur ein Chromosom für jede Art. Alle andern (3 in einer Klasse — 9 in der andern) lösen sich im Zellensaft auf. Auf diese Art und Weise entstehen Spermatiden, von denen ein jedes nur ein Chromosom enthält. Die eupyrenen Spermatiden und natürlich auch die von ihnen abstammenden Spermien enthalten sieben Chromosome.

Fasciolaria hat, wie *Paludina* und manche andre Prosobranchier, zwei Sorten von Spermien und ihrer Struktur nach zu schließen kann wenig Zweifel darüber sein, daß sie den oligopyrenen und eupyrenen Spermien, die MEVES bei *Paludina* so schön beschrieben hat, entsprechen. Was der Grund für diesen Dimorphismus ist, ist mir gar nicht klar. Aber meiner Ansicht nach ist es nicht unmöglich, daß eine Form, wie *Fasciolaria*, welche ihn in ihren Spermien so ausgesprochen aufweist, ihn auch in ihren Eiern haben kann.

Daß *Fasciolaria* zwei Sorten Eier besitzt, die in ihren Reaktionen mit Spermien, und in ihrem Schicksal sehr verschieden sind, ist zweifellos. Sind diese Verschiedenheiten auf ovogenetische Vorgänge analog den spermatogenetischen von *Paludina* zurückführbar, so wären die sterilen Eier von *Fasciolaria* — die »kernlosen Eier von *Neritina*«, und die unregelmäßigen Furchungen der Eier von *Purpura* erklärt. Sollten weitere Untersuchungen diese Hypothese bestätigen, dann wäre die Entstehung des Kannibalismus nicht in der Zuchtwahl seiner kleinen Anfänge, sondern in Vorgängen, die mit ihnen korrelativ verbunden sind, zu suchen.

Die Entwicklung der Excretkörper und des Velums.

Die Excretkörper, auch »äußere Excretzellen« genannt (CONKLIN), oder »Subvelarmassen« (OSBORNE) und »Urnieren« in der deutschen Literatur, beginnen bei *Fasciolaria* sehr früh, und sind auffallend nicht nur wegen ihrer Größe und Lage, sondern weil sie eine sehr wichtige Rolle im Stoffwechsel der Larve spielen.

Die frühesten Stadien wurden schon beschrieben (Fig. 3, 5 *Ex.K*).

Das nächste Stadium, das ich in meinem Material fand, zeigte die Excretkörper als zwei hohle Schwellungen, zwischen denen sich der Mund befindet (Fig. 6, 7, 8 *Ex.K*). Teile dieser Schwellungen stammen von den großen Zellen, welche die Seiten des Zylinders bildeten (Fig. 5 *Ex.K*), her, andre von danebenliegendem Ectoderm. Die Zellen haben jetzt nicht mehr die deutlichen Zellwände, sondern erscheinen wie ein Syncytium, in dem Vacuolen und Nuclei unregelmäßig verteilt sind.

Eingehendere Beobachtung zeigt, daß ein mehr oder weniger definitives Verhältnis zwischen den Nuclei und den Vacuolen existiert, da die größten dieser letzteren meistens neben den Kernen liegen.

Ein vergrößertes Bild eines Teiles des rechten Excretkörpers der Larve in Fig. 8 ist in Fig. 13 wiedergegeben. Das Chromatin der Nuclei ist in kleine Körnchen verteilt, die zu einem Netzwerk um den Nucleolus herum verbunden sind. Rechts unten zeigt sich ein Nucleus in einer der Vacuolen eingebettet. Dieser Zustand ist der Übergang zu den folgenden Veränderungen.

Fig. 9 und 12, 14 und 15 zeigen, daß die Excretkörper viel bestimmter rechts und links vom Munde abgegrenzt sind. Auch zeigen sich jetzt Zellwände; eine größere Anzahl kleiner, dagegen gar keine großen Vacuolen. Das Verschwinden der großen Vacuolen scheint dadurch hervorgebracht zu sein, daß die Nuclei, die schließlich eine centrale Stellung in den Zellen einnehmen, durch die Vacuolen hindurchpassieren und sie auf diese Art und Weise in kleinere zerteilen (Fig. 14).

Über das Verschwinden und Wiederauftauchen der Zellwände bin ich mir nicht klar. Ich möchte jedoch darauf hinweisen, daß sie wahrscheinlich in dem früheren Stadium durch den großen Intracellulardruck, den die großen Vacuolen erzeugten, so dünn gestreckt wurden, daß sie unsichtbar wurden; daß aber beim Zerstückeln der großen Vacuolen, und dem Auslaufen der kleineren, der Cellulardruck so verringert wurde, daß sie sich schließlich wieder zeigten. Diesen Prozeß des Auslaufens der kleineren Vacuolen habe ich nicht gesehen, jedoch scheint es mir möglich, durch die große Zunahme der kleinen Körnchen, die sich zu dieser Zeit im Zellsaft zeigen, daß solches stattfindet. Diese kleinen Körnchen, wie ich später noch zeigen werde, scheinen von den Oberflächen der Vacuolen herzustammen.

Nachdem die großen Vacuolen zerteilt sind, und die Nuclei ihre centrale Stellung in den Zellen genommen haben, haben die Excretkörper eine Form, wie in Fig. 9 und 12 gezeigt ist. In vielen Fällen sind sie durch die Ingestion der Eier nicht wahrnehmbar (Fig. 10). In Fig. 9, die von einer gefärbten Larve verfertigt wurde, zeigt es sich, daß jeder Excretkörper unten um seine Basis herum einen Ring von Nuclei hat. Zellwände sind nicht zu sehen. Die Zellen des Ringes, die am weitesten von den Basen der Excretkörper entfernt sind, nehmen an der Bildung des Velums teil, während die, welche der Basis am nächsten stehen, an der Weiterentwicklung der Excretkörper beteiligt sind. Die Beschreibung dieses Vorganges wird am besten in zwei Absätzen gegeben — die Veränderungen in dem Organ als ein Ganzes — und die Veränderungen, die sich in den einzelnen Zellen abspielen.

Die Veränderungen im Organ als Ganzes.

Ein horizontaler Schnitt durch die Excretzellen einer Larve im Alter derjenigen in Fig. 12 ist in Fig. 16 abgebildet. Der Schnitt zeigt die dünne Membran des Kopfbläschens (*Kpf.bl*), welche die zwei Außennieren vorn verbindet. Nach hinten ist der Schnitt offen. Die Außennieren sind zu dieser Zeit aus einer einzigen Lage ovaler Zellen gebildet, deren Inhalt in bestimmte Regionen geteilt ist (Fig. 17). Nach außen zu befinden sich die Kerne, welche in einer leicht färbbaren Mischung von Körnchen und Vacuolen eingebettet sind. Unter diesen färbbaren Substanzen ist klares und sehr fein granuliertes Cytoplasma. Kleinere Zellen, die sich diesen größeren seitlich anschließen, zeigen etwas verschiedene Struktur (Fig. 18).

Die weitere Entwicklung der Außennieren besteht darin, daß sie sich abrunden, wodurch sie in hohle Näpfchen verwandelt werden. Solch ein Näpfchen von der dem Embryo zugewandten Seite ist in Fig. 19 abgebildet. Die Zellen, durch die es mit der Wand des Embryos verbunden ist, unterscheiden sich durch ihre Größe und Form von den andern (*Verb.Rg*). Ein Vergleich mit Fig. 9 und 12, in denen die Außennieren an Ort und Stelle liegen, wird ihre Form klar machen.

Durch das Fortfahren des Abrundungsprozesses werden die Verbindungszellen mehr und mehr unter die Außennieren gezogen, und schließlich kommen die der entgegengesetzten Seiten nahe nebeneinander zu liegen. Diesen neuen Zustand zeigt Fig. 21, in welcher ein Querschnitt durch den Ring letzteren als zwei Kolben erscheinen läßt (*Verb.Rg*); auch verlieren diese Zellen ihre deutlichen Zellwände, wodurch die Kerne in eine rosettenartige Lage kommen, in der sie durch klare Räume voneinander geschieden sind.

Mit dem Vorschreiten des Abrundungsprozesses werden diese Kolben von Verbindungszellen schließlich zum Verschmelzen gebracht, und bilden auf diese Art und Weise einen Pfropfen (Fig. 22 *Pfrpf*). In diesem Pfropfen, wie in den Kolben, sind Zellwände schwer zu erkennen. Die Kerne sind entweder in einer soliden granulierten

Grundsubstanz irregulär eingebettet, oder am äußeren Ende des Pfropfens zu einer Reihe angeordnet, in der sie mit klaren Räumen abwechseln.

Unter stärkeren Vergrößerungen ist dieser Wechsel zwischen Kernen und klaren Räumen nicht so auffallend (Fig. 23), da die letzteren nicht nur von unregelmäßiger Form sind, sondern viel häufiger, als sie unter schwächeren Vergrößerungen zu sein scheinen. Bei diesen stärkeren Vergrößerungen zeigt es sich, daß das Chromatin in den Kernen unregelmäßig um die in einem klaren Raum vorkommenden Nucleolen zerstreut ist. Viele kleine klare Vacuolen kommen in den Kernen vor. Noch stärkere Vergrößerung zeigt, daß die klaren Zonen um die Nucleolen durch kleine von ihnen ausgehende Fortsätze durchsetzt sind (Fig. 24).

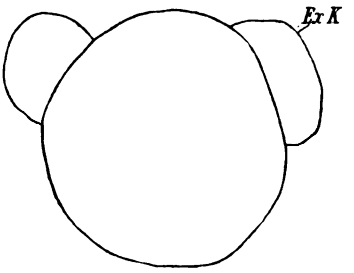
Ich konnte die Geschichte dieser Zellen nicht weiter in meinem Material verfolgen, doch glaube ich aus mehreren Gründen, daß sich schließlich die meisten in gewöhnliche Excretzellen verwandeln, die zwischen die außen gelegenen Zellen Fortsätze senden, und schließlich den ganzen Hohlraum der Außennieren ausfüllen. Ich glaube dies, weil in späteren Stadien überhaupt kein Hohlraum in den Außennieren existiert, und weil die inneren Zellen mit der Außenwelt durch feine Fortsätze verbunden sind, an deren Enden die Kerne sich befinden (Fig. 26 *Ftstz*).

Von dem Momente ihres Entstehens bis zum Abfalle der Außennieren ist ein Teil dieses Kolbens direkt mit dem Ectoderm des Embryos verbunden (Fig. 21, 22).

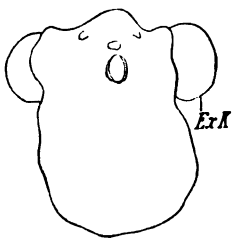
Bobretzky (77[2]) hat einen analogen Fall beschrieben, und Conklin (97) sagt von der Entwicklung der Außennieren bei *Crepidula*: »In early stages these cells form a part of the ectodermic layer, but as the embryo grows older, they grow more prominent, and the whole mass is constricted at the base, so that it becomes pear-shaped, the narrower end being attached to the embryo and the larger end being distal. The surrounding ectoderm cells crowd in at the neck of this constriction, and work their way entirely beneath these excretory cells.«

McMurrich (86[1]) fand überhaupt keine Membran unter den Außennieren von *Fascicolaria*, und Conklin hat darauf hingedeutet, daß dies daher käme, daß McMurrich sich mit den früheren Stadien beschäftigte, während Bobretzky die späteren Stadien seiner Form untersuchte. McMurrich jedoch arbeitete an früheren Stadien von *Fulgur*, und den späteren von *Fasciolaria*. Daher scheint es mir wahrscheinlicher, daß er die Membran nicht fand, weil sie in älteren Embryonen ganz außerordentlich dünn wird.

Gleichzeitig mit den schon beschriebenen Veränderungen finden andre statt, als deren Resultat die Nieren eine ganz andre Lage bekommen. Der Embryo nimmt zu dieser Zeit sehr stark an Größe zu — nicht nur durch Schlucken einer großen Anzahl Eier, sondern durch wirkliches Wachstum. Diese Vorgänge bewirken, daß die Larven speziell an der Basis des Kopfbläschens sehr breit werden, so daß die Außennieren von vorn nach den Seiten verschoben werden (Textfig. 1). Später werden durch große Tätigkeit in der Anlage des Velum, und durch weiteres Wachstum des ganzen vorderen Endes, die Außennieren nach oben geschoben (Textfig. 2).

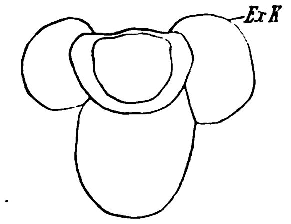


Textfig. 1.

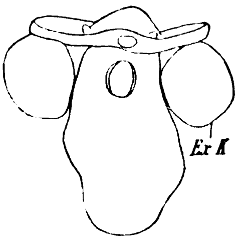


Textfig. 2.

Zu dieser Zeit wird auch in dieser Region das Velum auffallend, und mit seinem Wachstum seitwärts werden die Außennieren auch nach den Seiten zu mitgeschleppt (Textfig. 3). So kommt es denn schließlich zustande, daß sie an den unteren Seiten des Velum hängen, wonach sie von Osborn (86[2]) Subvelarmassen genannt worden sind (Textfig. 4).



Textfig. 3.



Textfig. 4.

In dieser Lage bleiben dann die Organe bis zu Ende des Larvenlebens. Ist dieses vorbei, so degenerieren sie — die Kerne der Zellen lösen sich auf, und in den meisten Fällen werden sie abgestoßen, ehe das Velum resorbiert wird. Es kommt aber eine große Anzahl von Ausnahmen vor, da in manchen Fällen ein viel intimeres Verhältnis zwischen Velum und Außennieren besteht. Fig. 27 ist ein typischer Fall. In dieser Niere wurde, nachdem ihre primitive Höhle durch den Kolben ausgefüllt wurde, das Velum selbst noch hineingezogen und dermaßen zusammengequetscht, daß sein eignes Lumen vernichtet wurde. In diesen Fällen wird das Velum oft mit den Außennieren als ein Stück abgeschnürt.

Diese intime Verbindung zwischen Velum und Niere erinnert an die »Ansae«, die SARASIN (82²) bei *Bithynia tentaculata* beschrieb. In dieser Form ist das Verhältnis beider noch ein viel intimeres. In Wirklichkeit ist das Velum selbst bei *Bithynia* zu einer Art Excretapparat modifiziert worden.

Veränderungen in den einzelnen Zellen.

Während die Außennieren sich, wie schon beschrieben, entwickeln, erleiden die einzelnen Zellen wichtige Veränderungen. Die Zellen sind von Vacuolen erfüllt, mit dazwischen zerstreuten Granula, welche einen mehr oder weniger deutlichen Ring entlang der Zellwände bilden (Fig. 15 *gr.Rg*).

Nachdem die Kerne ihre centrale Stellung in den Zellen angenommen haben, sind sie charakterisiert durch ihr fein granuliertes Chromatin und durch ein oder zwei Nucleolen, welche sich verschieden färben lassen und von einer klaren Zone umgeben sind. Große intercellulare Räume kommen auch vor, wahrscheinlich als eine Folge der schon erwähnten Schrumpfung der Zellen. Später jedoch rücken die Zellen näher aneinander an und die intercellularen Räume verschwinden (Fig. 20). In diesem Zustande treten die Zellwände viel mehr, wie in irgend einem früheren Stadium, hervor. Es kommt dies daher, daß die Zellwände nicht verschmelzen, sondern nur durch die Annäherung der anliegenden Zellen in der Dicke verdoppelt sind. Außerdem liefert jede Zelle einen Ring von Granula, welcher die innere Fläche ihrer Wände, wo diese in Berührung mit denen der Nachbarzellen sind, bekleidet. Ein Gang von Zelle *A* zu Zelle *B* würde daher ein Passieren durch den Granularing von *A*, die Zellwand von *A*, einen kleinen intercellularen Raum zwischen Zelle *A* und Zelle *B*, die Zellwand von *B* und den Granularing von *B* einschließen.

Nachdem die Zellen polygone Form angenommen haben, findet Amitosis statt, mit der Bildung polynucleierter Zellen mit drei bis fünf Nuclei (Fig. 20). Dieser Vorgang der Amitosis ist detailliert in Fig. 28—39 dargestellt.

Zwei Eigentümlichkeiten in diesen Amitosen sind auffallend, die Formen der Kerne und das Verhalten der Nucleolen. In bezug auf die ersteren ist es klar, daß die Unregelmäßigkeit im Umriß teilweise eine Folge der Vacuolen ist, welche auf die Kernwandungen drücken (Fig. 33, 34, 37). In Fällen, in welchen die Kerne einen Prozeß der Zusammenziehung durchmachen (Fig. 31, 32, 33, 34, 37), ist es durchaus nicht sicher, wie viel von der Teilung auf innere Tätigkeiten des Kernes, und wie viel auf Druck von außen zurückzuführen ist. In bezug auf die Nucleolen ist es ganz augenscheinlich, daß sie in keiner ursprünglichen Beziehung zu den Kernteilungen stehen, da in manchen Kernen, welche keine Anzeichen einer Teilung aufweisen, zwei (Fig. 28, 29) vorhanden sind; in andern, welche noch in Teilung begriffen sind, drei vorkommen, in manchen Fällen von sehr verschiedener Größe (Fig. 31, 32, 34, 37), während einige Kerne keine Nucleolen zeigen (Fig. 36, 39). Die Nucleolen beweisen klar, daß die Generalisierung bekannt als »REMAKS Schema« nicht zu diesen Kernteilungen paßt.

Ich bin nicht imstande gewesen, einen einzigen Fall zu finden, in welchem der Nucleolus in Teilung begriffen war, und der Schluß, daß eine solche Teilung entweder niemals stattfindet, oder wenigstens nicht in klarer Beziehung zu den Teilungen des Kernes steht, ist weiterhin gestützt durch solche Fälle, in welchen drei Nucleolen sehr verschiedener Größe und weit getrennt in solchen Kernen gefunden werden, welche ihre erste Amitose noch nicht vollendet haben. Ebendasselbe ist durch solche Fälle, in welchen sich Kerne auf einmal in mehr als zwei Fragmente teilen, illustriert (Fig. 20). Besonders aus diesen Fällen bin ich geneigt zu schließen, daß Nucleolen neu entstehen können.

Die amitotischen Teilungen fahren fort, bis die ursprünglichen Kerne in vier bis fünf Teile geteilt sind, welche in manchen Fällen in den Zellen weit verbreitet werden. In den Fällen, in welchen die Kerne sich sofort in drei oder mehr Fragmente teilen, bleiben die Stücke einige Zeit nahe beieinander (Fig. 20). Gewöhnlich aber trennen sich die Kerne und nehmen an Größe zu, so daß sie, zusammenaddiert, größer sein würden wie der Mutterkern, aus welchem sie entstanden sind.

Es ist interessant in dieser Beziehung, daß MCMURRICH (86[1]) nur einen Nucleus in den Ausscheidungszellen beschrieben hat, während

Osborn (86[2]), in demselben Bande, in welchem McMurrichs Artikel erschien, angibt, daß keine Kerne in den »sub-velar« Zellen der *Fasciolaria* existieren. Indessen sagt Osborn in einer kürzlich veröffentlichten Mitteilung (04): »In den äußeren Enden der Zellen findet man starke Anzeichen von Amitosis.«

Hiernach bin ich nicht sicher, ob er den Prozeß wirklich gesehen, oder nur gute Gründe, ihn zu vermuten, hat.

Die Kerne, welche aus amitotischen Teilungen hervorgegangen sind, sind verhältnismäßig groß und sehr günstige Objekte für das Studium. Mit den höchsten Vergrößerungen, welche ich benutzte (Obj. 12, Oc. 8, 12, 18), war ich imstande, in Schnitten zu sehen, daß die jeden Nucleolus umgebende Zone nicht einfach ein runder Raum, sondern daß wirklich ihre Außenlinie sehr unregelmäßig ist, indem sie sich nach außen in fein verjüngten Fortsätzen in den Körper des Kernes verlängert (Fig. 40, 41, 42). Diese Zonenfäden sind durch feine Granula begrenzt, welche in dem Kernsaft suspendiert sind. Sie sind viel kleiner wie das Chromatin des nuclearen Reticulum, geben aber dieselben Reaktionen mit Farbstoffen. Daraus vermute ich, daß sie wirklich Chromatin in einem Zustande feiner Verteilung sind.

An jeder Seite seiner Basis hat ein Fortsatz einen größeren Chromatinklumpen, welcher ein Teil des Chromatinnetzwerkes ist. In vielen Fällen enden gewisse Fäden distal in Anschwellungen (Fig. 40 *Vac*), welche manchmal auf der dem Nucleolus benachbarten Seite zusammengeschnürt sind. Diese Konstriktion mag durch den Druck der großen Chromatinklumpen verursacht sein. Manche Kerne enthalten Vacuolen (Fig. 40 *Vac'*) (siehe auch viele vorhergehende Zeichnungen), während andre Vacuolen zeigen, welche nach außen in die Zelle ausbrechen (Fig. 41, 42 *Vac''*). In einem Falle (Fig. 42 *Vac''*) wurde gesehen, daß eine solche Vacuole direkt von der Extranucleolarzone in den Zellkörper entschlüpfte. In allen andern Fällen, in welchen entschlüpfende Vacuolen beobachtet wurden, schienen sie von andern Orten in den Kernen zu kommen. Das Arrangement der Chromatinklümpchen an der Basis der Fortsätze erklärt, warum die Zonen in ganzen Präparaten unter schwacher Vergrößerung in runder Form erscheinen, denn die Fortsätze zwischen den Chromatinmassen sind dünn und farblos, daher unsichtbar unter Bedingungen, welche das Chromatin allein zeigen. Aber die Stellung der Granula kann auch über die unregelmäßige Form der Extranucleolarzone selbst Aufschluß geben. Denn wenn wir uns eine flüssige Kugel als nach außen in allen Richtungen vom Nucleolus vorstrebend vorstellen, würden Hindernisse irgendwelcher Art, nachdem sie so viel als möglich nachgegeben hätten, sie aufhalten, ausgenommen in den Richtungen des geringsten Widerstandes, welche sich natürlich zwischen den Granula befinden würden. Daß die Vacuolen Flüssigkeit enthalten, ist nach später folgenden Betrachtungen wahrscheinlich; daß die großen Chromatingranula mehr Widerstand bieten, wie der gewöhnliche, fein granulierte Kernsaft, ist, glaube ich, eine gerechtfertigte Annahme, nicht nur als Folge ihrer relativen Größe und Dichte, sondern auch, weil das gröbere Chromatin zu einem bestimmten Netzwerk verbunden ist, welches sicher als mehr widerstandsfähig angesehen werden kann, als der Kernsaft, in welchem es suspendiert ist.

Eine einzelne Vacuole ist in Fig. 43 stark vergrößert gezeigt. Ihre Oberfläche scheint ein mit kleinen Granula bedecktes Häutchen zu sein; die Granula halte ich für identisch mit den kleinen Granula des Kernsaftes, in welchem die Vacuole früher suspendiert war. Wenn eine solche Vacuole platzen sollte, und der flüssige Inhalt austreten, würde eine große Anzahl von Granula zurückbleiben. Dieses Platzen von Vacuolen würde sowohl die Entstehung des Granularinges erklären, von dem schon öfter die Rede war, als auch die Verminderung des intracellulären Drucks, durch welchen in einem früheren Stadium die Zellwände plötzlich sichtbar werden. Das Innere einer mit Reagentien behandelten Vacuole ist von einem zarten, fast unsichtbaren Netzwerk, dem Coagulum, durchsetzt (Fig. 43), dies und die Tatsache, daß sich das Innere einer Vacuole nicht färben läßt, sind meine Hauptgründe für die Annahme, daß sie Flüssigkeit enthalten.

Ehe ich zu den Veränderungen, welche die Zellen außer durch Amitosis erleiden, oder zu meiner Auffassung der vorgelegten Tatsachen übergehe, will ich in Kürze die Natur des Nucleolus betrachten. MONTGOMERY (99) hat in seiner Abhandlung darauf aufmerksam gemacht, daß Gebilde von sehr verschiedenen Eigenschaften verschiedentlich und nachlässigerweise Nucleoli genannt worden seien. Später (02) hat HOFFMANN kurz die verschiedenen Klassen von Körpern, welche Nucleoli genannt worden waren, verzeichnet. Diese Klassen sind: 1) Ansammlungen von Chromatin in größerer Masse, wie in den gewöhnlichen Chromatingranula, 2) kleine, klare vacuolenähnliche Gebilde, die sich schwach färben lassen (Paranucleoli nach MONTGOMERY), 3) runde Körper in Keimbläschen, welche außer Plastin alles Chromatin des Kernes enthalten, 4) Körper, welche sich verschieden von dem Chromatin färben, die sogenannten wahren Nucleolen oder Plasmosomen.

Der Nucleolus, welchen wir betrachtet haben, ist keine Anhäufung von Chromatin, da er eine davon verschiedene Färbungsreaktion hat.

Dies schließt die erste und dritte Möglichkeit aus.

Die zweite Möglichkeit fällt auch weg, weil der Nucleolus weder eine Vacuole noch ein Paranucleolus ist, was durch seine Dichte und seine Affinität für Färbemittel bewiesen ist. Nur die vierte Möglichkeit bleibt übrig, nämlich die, daß er zur Klasse der Plasmosomen oder wahren Nucleolen gehört. Hierher gehören die Nucleolen in den Außennieren der *Fasciolaria*, wie die, welche Hoffmann (02) in den großen Makromeren der *Nassa mutabilis* beschrieben hat. In dieser Art scheinen die Nucleolen eine sehr wichtige Rolle in der Ernährung des Embryo zu spielen. In *Fasciolaria* nehmen die Nucleolen, wie später klarer werden wird, auch Teil an dem Metabolismus des Embryo, obgleich die geleistete Arbeit nicht so klar angezeigt ist, wie in Hoffmanns Zeichnungen der *Nassa*.

Die andern Veränderungen, welche in den Zellen der Außennieren stattfinden, teilen sich in drei Klassen: Veränderungen der Größe, der Form und des Inhalts. Gewöhnlich wachsen die Zellen stark in die Länge, ohne eine entsprechende Vergrößerung der Breite. Dies führt zu langen Zellen, welche infolge der Zusammendrängung, unter der sie sich entwickeln, prismatisch werden (Fig. 44). Solche Zellen, welche vom Pfropfen abstammen, und welche das Innere der ursprünglichen Käppchen ausgefüllt haben, nehmen auch stark an Größe zu und senden Fortsätze bis nach außen zwischen die andern Zellen. Dies ist besonders wahrnehmbar in Querschnitten (Fig. 26 *Ftstz*). Diese sekundären Ausscheidezellen zeigen auch, wie die primären, außer den Veränderungen in Größe und Form, Veränderungen ihres Inhalts. Während im Anfang die sekundären Zellen nicht besonders körnig, und die primären nur sehr feinkörnig waren, sind in den unteren klaren Räumen (Fig. 16, 17, 18) diese beiden Arten von Zellen in ihrem voll entwickelten Zustande überall grobkörnig. Die groben Granula sind in einer Art Reticulum geordnet, welches ein sehr unruhiges Muster hat. An den Enden der Zellen ist das Muster durch die Gegenwart von Vacuolen und Nuclei unterbrochen (Fig. 26, 27, 45). Eine typische Zelle aus der Mitte der voll entwickelten Außenniere genommen, ist außer durch ihre prismatische, verlängerte Form, durch eine Art Haube charakterisiert, in welcher sich Kerne und Vacuolen befinden. Die Kerne sind schon beschrieben worden und es ist ferner nichts zur Beschreibung der Haube hinzuzufügen, da diese weiter nichts ist als die obere Fläche der Zellen. Ein Gegenstand, der schon mehrere Male erwähnt wurde, zeigt sich besonders gut sowohl in Zupfpräparaten als in Schnitten — der Granularing. In ganzen Zellen wird dieser Ring an dem unteren Rande der Haube gesehen, wo er sich etwas über der prismatischen granulären Region unter ihm erstreckt. Dieser Ring ist im Schnitte in Fig. 45 *gr.Rg* gezeigt.

Wie schon erwähnt, degenerieren die Außennieren gegen Ende des Larvenlebens. Dieser Prozeß führt zum Verschwinden der Kerne und einer Verminderung in der Größe der Zellen, wodurch diese ihre eckige Form verlieren. Die Kerne verlassen ihre centrale Lage und bewegen sich gegen die Zellwände, wo sie verflachen, sehr dünn werden, und schließlich sich in Granula auflösen, ähnlich denen des Granularinges.

Die Verminderung der Größe der Außennieren ist nicht in erster Hand eine Folge vom Abstoßen von Zellen, obgleich dies stattfindet, sondern eher auf tatsächliche Reduktion der Größe der Zellen selbst zurückzuführen. Es scheint, als ob deren Inhalt nach dem Verschwinden der Kerne löslicher wird als wie zuvor. Schließlich werden entweder die Außennieren allein abgeworfen, oder es mag in den Fällen, in welchen sich nähere, als die gewöhnlichen, Beziehungen zum Velum entwickelt haben, auch dieses mit ihnen abgestoßen werden.

Sekundäre Außennieren.

Eine der überraschendsten Tatsachen, denen ich in meinem Studium des Embryo der *Fasciolaria* begegnet bin, ist die Anwesenheit in einer großen Anzahl von Larven mit sekundären Außennieren. Nicht nur, daß deren Vorkommen sehr unerwartet ist, man findet sie auch an ungewöhnlichen Plätzen. In manchen Fällen werden sie an der Unterseite des Velums, gerade hinter den großen Nieren gefunden. Hier erreichen sie etwa ein Drittel der Größe der primären Organe. In andern Fällen werden einzelne Zellen des postoralen Velums modifiziert (Fig. 46, 47 *ExK'*), und in noch andern (Fig. 48 *ExK'*) fand ich gewisse Zellen des Kopfbläschens bedeutend vergrößert und den Excretionszellen ähnlich. Bisweilen wurde Amitosis in diesen Hilfsnieren beobachtet. Das Vorkommen dieser Zellen in verschiedenen

Plätzen, besonders im Bereich des postoralen Velums zeigt, daß nicht viel Grund zu der Ansicht, welche manche Autoren aussprechen, vorliegt, daß eine Zelle, weil sie eine Zelle des Velum ist, nicht auch eine Excretionszelle sein könnte.

Die Bedeutung der Amitosis.

Wilson (02[2]) hat eine kurze Übersicht der Literatur über amitotische Kernteilungen gegeben, und hat auch den allgemeinen Stand unsres Wissens in bezug auf den Zweck dieses ungewöhnlichen Prozesses besprochen. Das allgemeine Resultat früherer Studien ist, daß Amitosis, ausgenommen in Fällen, wo sie auf pathologische Zustände zurückführbar ist, mit einem hohen Grade von Spezialisierung verbunden ist, und daß Zellen, auf diese Weise spezialisiert, auf dem Wege zur Degeneration sind. Diese Ansicht wurde von Flemming ausgesprochen und fand Stütze durch die Untersuchungen von Ziegler und vom Rath. Ziegler zeigte, daß amitotisch sich teilende Kerne durch ihre Größe und die Tatsache charakterisiert sind, daß sie oft sich im Zustande sehr aktiver Secretion oder Assimilation befinden. vom Rath kam zu dem Schluß, daß, »wenn einmal eine Zelle amitotische Teilung durchgemacht hat, sie ihr Todesurteil empfangen hat; sie mag nun wirklich für einige Zeit fortfahren, sich durch Amitosis zu teilen, geht aber unabweislich schließlich zu Grunde« (Wilson).

Spätere Forschungen von Meves, Preusse und Pfeffer haben gezeigt, daß dieser Schluß zu extrem ist. Ganz kürzlich hat Osborn (04) Tatsachen ans Licht gebracht, welche später besprochen werden sollen, die anscheinend nicht in Übereinstimmung mit der Lehre sind, daß Amitosis ein Zeichen von seniler Degeneration der Zellen sei. Conklin (03) auf der andern Seite ist zu dem Schluß gekommen, daß amitotische Teilungen in den niederen Follikelzellen des Heimchens (*Gryllus pennsylvanius*, *Gryllus abbreviatus* und *Gryllus domesticus*) Erscheinungen der Senilescenz sind.

Der Fall von *Fasciolaria* zeigt klar, daß die amitotischen Teilungen Begleiter eines hohen Grades von vegetativer Tätigkeit sind. Nicht allein zeigt sich dies durch den fein verteilten Zustand des Chromatins, sondern auch durch die Entstehung von Vacuolen in den Kernen und deren Übertritt in die Zellen. Wie vorher erwähnt bleibt es zweifelhaft, ob die Nuclei ganz aus eignem Antriebe sich teilen, oder ob die Teilungen zum Teil Folge des Druckes der Vacuolen sind. Ob der eine oder der andre Grund oder beide vorliegen, macht keinen Unterschied im Endresultat, denn die Oberfläche, auf welcher Metabolismus stattfinden kann, ist sehr durch die Teilungen vergrößert, ohne Rücksicht auf die Gegenwart oder Abwesenheit teleologischer Faktoren. Ich glaube daher, daß in unserm Falle Amitosis mit einem Grade von secretorischer Tätigkeit verknüpft ist (vielleicht reciprok).

In bezug auf die Lehre der Zellen-Senilescenz möchte ich mir ein Urteil vorbehalten. Wie OSBORN (04) gezeigt hat, kann Amitosis in Embryonalzellen nicht gut mit Altersschwäche verknüpft sein. VOM RATHS Spruch, daß eine Zelle, welche amitotische Teilung erlitten hat, zum Tode verurteilt sei, ist nicht nur in einem Spezialfall durch PFEFFERS Experimente an *Spirogyra* als unrichtig erwiesen worden, sondern er ist als Verallgemeinerung kein sehr wichtiger Beitrag zur Wissenschaft, da jede Zelle früher oder später zugrunde gehen muß. Wenn wir dem Worte »verurteilt« einen besonderen Sinn unterlegen sollen, so muß es der sein, daß die Zellen, auf welche er sich bezieht, ihren Lebenspfad sicherer vorgeschrieben haben, als wie es gewöhnlich der Fall ist. Mein Studium der Amitose in den Außennieren der *Fasciolaria* hat mich überzeugt, daß der Prozeß in diesem Fall nicht mit Altersschwäche der Zellen verbunden ist, da sie sehr früh in dem Leben der betreffenden Zellen auftritt.

Wirklich folgt die längste Periode der Tätigkeit, durch welche diese Zellen passieren, der Amitose, und geht nicht vorher. Wenn diese Art der Kernteilung ein Zeichen von Altersschwäche wäre, würde man erwarten, daß es die letzte Handlung der Kerne sein würde, wie dies bei den Teilungen der Keimbläschen in den verschluckten Eiern der Fall ist.

Funktion der Außennieren.

Die Autoren, welche die Funktion der Außennieren beschrieben haben, fallen in zwei Gruppen: in die, welche annimmt, daß diese Organe Renalorgane sind, und in die, welche leugnet, daß sie ausscheidende Arbeit verrichten. Einer der ersten, der sich zugunsten einer excretorischen Tätigkeit aussprach, war SELENKA (72^1), welcher *Calyptrea sinensis* bearbeitete. BOBRETZKY (77^2) kam zu einem ähnlichen Schluß durch sein Studium der Embryologie von *Nassa*, *Natica* und *Fusus*. RABL (79) indessen leugnet, daß die entsprechenden Zellen von *Planorbis* als Excretionsorgane wirken, und scheint seine Meinung auf die Tatsache zu basieren, daß sie ein Teil des Velum sind. SARASIN (82^2) fand eine besonders nahe Beziehung zwischen

Velum und diesen Renalzellen (zusammen die Ansae) in *Bithynia*, war aber aus diesem Grunde nicht geneigt, ihre excretorische Tätigkeit zu bezweifeln. McMurrich und Osborn (86) kamen zu ähnlichen Schlüssen, der erstere in bezug auf *Fulgur* und *Fasciolaria*, der letztere nur in bezug auf *Fasciolaria*. Erlangers (92) Studium von *Bithynia* brachte ihn in Übereinstimmung mit Rabl. Erlanger schlug vor, diese Zellen, anstatt von ihrer Ausscheidungsfunktion zu reden, für den Leberzellen ähnlich zu halten, daß sie Lagerhäuser für Reservenahrungsstoffe seien, welche später in der Entwicklung verbraucht werden. Angesichts der großen Ähnlichkeit, welche zwischen den großen Entodermzellen der hinteren Wandung des Schlundes bei *Fasciolaria* (später zu beschreiben), und den Außennieren besteht, ist Erlangers Meinung zum mindesten nicht unnatürlich. Conklin (97) kommt zu dem Schluß, daß die entsprechenden Zellen der *Crepidula* Excretionsorgane seien, und er scheint mir der erste gewesen zu sein, welcher andre Gründe für seine Meinung, als die Anwesenheit von Konkrementen und Vacuolen, hatte; denn er gibt an, daß die äußeren Excretionszellen, statt sich mit Hämatoxylin-Königspurpur zu färben, eine dunkle Karminfarbe annehmen, wenn sie mit diesem Reagenz behandelt werden. Da diese Karminfarbe auftritt, wenn Hämatoxylin mit einer schwachen Säure behandelt wird, kommt Conklin zu dem Schluß, daß die Excretionszellen ein saures Secret enthalten.

Von der Entwicklung und der Struktur der äußeren Excretionskörper der *Fasciolaria* ist die Ansicht, daß diese Zellen am Metabolismus kräftig beteiligt sind, gewiß gerechtfertigt, aber daß sie als Renalorgane funktionieren, folgt nicht notwendigerweise aus morphologischen Gründen.

Ich bin imstande gewesen, sicheren Beweis zu erbringen, daß sie Secretionsorgane sind. Das Material, auf welches ich meine Schlüsse basiere, wurde durch eine chemische Analyse erbracht, welche mein Vater so freundlich war für mich zu machen. Da diese Analyse nicht nur in ihrer Beziehung zu dem Gegenstand unsrer Diskussion interessant ist, sondern auch als ein Beispiel davon, was mit kleinen Mengen von Material zu erreichen ist, gebe ich sie etwas ausführlicher wieder.

Im Sommer 1903 entnahm ich mehrere hundert Außennieren von *Fasciolaria*-Larven, extrahierte sie in Chloroformwasser und sandte die Lösung nach Baltimore.

1) Ein Kubikzentimeter dieser Lösung wurde mit alkalischer

Permanganatlösung destilliert, und der totale Stickstoff als 3504 g per Liter gefunden.

2) Da die Embryonen in einer eiweißhaltigen Substanz schwimmen, und da etwas von dieser unvermeidlich mit den Außennieren in das Chloroformwasser gelangt war, war es nötig eine Korrektur für diese Fehlerquelle einzufügen. Dies geschah durch Coagulation des echten Eiweißes in 1 ccm Flüssigkeit durch Behandeln mit Essigsäure und Erhitzen. Nach Fällung des echten Eiweißes wurde das Filtrat wie vorher mit alkalischem Permanganat destilliert. Es waren nur noch 2262 g Stickstoff per Liter vorhanden.

Folglich war das echte Eiweiß als Stickstoff ausgedrückt die Differenz von 1242 g per Liter.

3) Der nächste Schritt war die Bestimmung des Stickstoffs, welcher gewöhnlich als »freies Ammoniak« angesprochen wird, d. h. welcher als Ammoniaksalz oder als Amine vorhanden ist, welche durch Alkali zerlegt werden. Zu diesem Zweck wurde 1 ccm der Flüssigkeit mit Natriumcarbonat destilliert. Es wurde gefunden: Stickstoff als »freies Ammoniak« 0099 g per Liter.

4) Der Zweck der nächsten Operation war festzustellen, ob Albumosen oder Peptone vorhanden seien. Hierfür wurde 1 ccm der Originallösung mit Natriumchlorid gesättigt, und mit Phosphor-Wolframsäure gekocht. Die Lösung wurde abfiltriert und das Filtrat mit Äthylalkohol versetzt, bis das Natriumchlorid sich ausgeschieden hatte. Dann wurde wieder filtriert und das Filtrat, wie vorher, mit Kaliumpermanganat behandelt. Das Resultat war 2273 g Stickstoff per Liter. Es war so bewiesen, daß weder Albumosen noch Peptone vorhanden waren. Die 2273 g dieses Experimentes sind für unsre Zwecke identisch mit den 2262 g des zweiten Experimentes. Wenn man von dem Totalstickstoff, wie zuerst gefunden, den Stickstoff abzieht, der als »freies Ammoniak« vorhanden ist, so verbleiben 2163 g per Liter, welche noch nicht identifiziert sind.

5) Wenn dieser Rest von 2163 g Stickstoff per Liter aus Urea oder Homologen besteht, so sollte theoretisch 1 ccm der Flüssigkeit 17 ccm Stickstoff Gas entwickeln, wenn er mit Natrium hypobromid behandelt wird. Tatsächlich wurde etwas weniger wie 2 ccm Gas erhalten. Da dies den Stickstoff, welcher das »freie Ammoniak« entwickelt, mit einbegreift, 0099 g per Liter, so können wir ruhig annehmen, daß die Postulate der Theorie erfüllt wurden und bestimmt aussprechen, daß die Gegenwart von Harnstoff oder Homologen in dem wässerigen Auszug der Außennieren der *Fasciolaria* bewiesen ist.

Die Resultate der Analyse sind in folgender Tabelle zusammengestellt[1]:

Ein Liter des wässerigen Auszuges der Außennieren enthält:	N als Eiweiß	1243 g
	N › freies Ammoniak	0099 ›
	N › Harnstoff oder Homologe	2163 ›
	Totaler Stickstoff	3504 g

Der Beweis, daß Ausscheidung stattfindet, war lange in meinem Besitz, ehe ich eine Idee davon hatte, wie die Ausscheidung der Stoffe tatsächlich stattfand. Hoffmann (02) fand Stützen für die Annahme, daß die Nucleolen der großen Makromeren von *Nassa mutabilis* den Prozeß der Excretion so vornehmen, daß sie Nahrung von den großen, mit Dotter beladenen Zellen aufnehmen, und sie in den Verdauungskanal überführen, in welchen die ventralen Enden dieser Zellen vorspringen. Die so ausgeschiedenen Stoffe, glaubt Hoffmann, treten als Rohprodukte auf einer Seite der Nucleolen ein und verlassen dieselben auf der andern in verändertem Zustande. Diese Meinung war auf die Beobachtung basiert, daß die Nucleolen immer eine zusammenhängende Front auf der Seite zeigen, auf welcher Rohmaterial sie zu erreichen erwartet werden kann, während sie auf den Seiten, nach dem Verdauungskanal hin, immer die Form eines Viertel Mondes, mit unregelmäßig gezackten Schneiden haben, als ob Stoffe aus ihnen austreten. In einigen Fällen wurden solche Stoffe wirklich gesehen. Während die Nucleoli der Außennieren der *Fasciolaria* unzweifelhaft in den Metabolismus eingreifen, bin ich nicht imstande gewesen, solche direkte morphologische Beweise zu finden, wie sie Hoffmann in *Nassa* nachgewiesen hat. Die Nucleolen der *Fasciolaria* haben keine konstante Stellung in bezug auf die Zufuhr von Rohmaterial, denn erstens sind sie viel weiter von der ursprünglichen Quelle des Rohmaterials entfernt — dem Verdauungskanal —, und ferner sind keine zwei Nucleolen in gleicher Entfernung von dieser Quelle. In Ermangelung von Beweisen, welche sich mit den von Hoffmann gelieferten vergleichen lassen, bin ich nicht imstande, mir über die Einzelheiten der Transformationen, welche durch die Kerne und Nucleolen bewirkt werden, eine Vorstellung zu machen.

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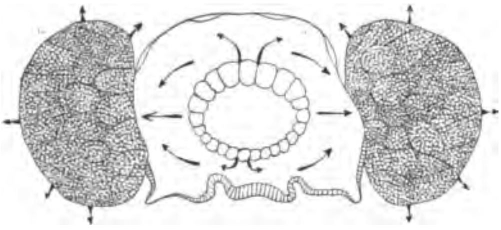
[1] Der normale Betrag von Stickstoff in Seewasser war in der Analyse vernachlässigt, da er sich in den angewandten kleinen Mengen nicht nachweisen ließ.

Alle Bestimmungen, außer der des freien Stickstoffgases wurden colorimetrisch mittels Nesslers Reagenz in Hehners Zylindern ausgeführt.

Osborn (04) berichtete in einer kurzen Abhandlung, daß die cuboidalen Entodermzellen der »Gastrula« von *Fasciolaria* Amitosis zeigen, und vermutet, daß diese Tätigkeit in Verbindung mit dem Verdauungsprozeß stattfindet. Die frühesten Stadien, welche ich besitze, und welche überhaupt Entoderm zeigen, sind keine Gastrulae in technischem Sinne, noch sind die Entodermzellen cuboidal. Sie sind, wie auf den vorhergehenden Seiten gezeigt wurde, spindelförmig und stark verlängert, was genau so ist, als man von einer Membran erwarten würde, welche so stark wie das Entoderm während der Periode des Kannibalismus gestreckt ist. Osborns »Gastrulae« sind wirklich »Kannibalen«, in welchen der definitive Mund längst durchgebrochen ist, und in welchen die Außennieren schon angelegt sind. Nach einer langen, vergeblichen Suche durch die frühesten Stadien, habe ich schließlich die von Osborn beschriebene Amitosis in dem Vorderende des Schlundes einer Larve gefunden, welche ein Velum hatte. In der vorderen Region des Schlundes sind die Zellen seiner hinteren Wand groß, vielkernig und stark granuliert (Fig. 49, 50, 51 *End.Ex*). An den distalen Enden sind Vacuolen. Die Kerne sind wahrscheinlich durch Amitosis entstanden. Einige dieser Zellen sind zerplatzt und ihr Inhalt fließt heraus. Sie ähneln stark den Zellen der Außennieren. Die Zellen der lateralen und ventralen Wände des Schlundes (Fig. 49) haben ein sehr verschiedenes Aussehen. Diese, anstatt lang und granuliert zu sein, sind von sehr unregelmäßiger Form, das Cytoplasma ist fast unsichtbar, und nahe an die Zellwände gedrängt, oder in unregelmäßigen Strängen. In dem Centrum haben manche der Zellen einen großen freien Raum, in welchem die Kerne liegen. Die Nucleolen dieser Kerne liegen öfters in freien Vacuolen, und in vielen Fällen sind weitere Vacuolen an andern Orten in den Kernen verteilt (Fig. 52). Von diesen sind viele im Prozeß amitotischer Teilung. Am Rande der lateralen und ventralen Wände des Schlundes scheinen manche Grenzzellen abgestorben zu sein, während andre deutlich im Absterben begriffen sind. Dadurch ist die Außenlinie höchst unregelmäßig gemacht, indem sie ausgefranst und mit Granula und Fragmenten von Zellen besetzt erscheint. Dieses Absterben der Zellen, deren allgemeines vacuolisiertes Aussehen, das Vorkommen von Amitose, und der fein verteilte Zustand des Chromatins in den Kernen zeigen alle an, daß die Zellen aktiv in den Metabolismus eingreifen, und die einfachste Vorstellung ist, daß sie, wie Osborn vermutet, am Verdauungsprozeß beteiligt sind.

Daß etwas aus diesen Zellen austritt, lehrt ihr Aussehen. Ich glaube daher, daß von ihnen im Laufe ihrer Tätigkeit als Verdauungszellen, Abfallprodukte ausgeschieden werden, welch letztere durch die Körperhöhle in die Höhle des Velum übertreten, durch die basale Membran in die Außennieren gelangen, und durch diese ausgestoßen werden. Die Textfig. 5 ist bestimmt, eine graphische Vorstellung dieses Prozesses zu geben.



Textfig. 5.

Homologien der Außennieren.

Während viele ältere Autoren, seit Ganin, die Außennieren der Prosobranchier erkannt, und ihnen Ausscheidungsfunktionen zugeschrieben haben, ist, glaube ich, ein tatsächlicher Beweis, daß wirklich Excretion stattfindet, nicht erbracht worden. Die bereits erwähnten Differenzen in bezug auf die Funktion dieser Organe sind auch von einer bedeutenden Diskussion über deren Homologien begleitet. So erklärt Salensky (72) die Außennieren der *Calyptrea sinensis* für homolog mit den »Urnieren« der Pulmonaten, und umgekehrt, homologisiert Fol (75) die Außennieren der Pulmonaten mit den Urnieren der Prosobranchier. Bobretzky (77[2]), indem er die Urnieren von *Nassa mutabilis*, *Natica* und *Fusus* beschrieb, schien die von Salensky aufgestellten Homologien angenommen zu haben. Bütschli (77[1]) aber, in seiner Arbeit über *Paludina vivipara* verwahrte sich sehr gegen diese Vergleiche. Er machte darauf aufmerksam, daß die Folschen Homologien sich auf ganz verschiedene Gebilde beziehen, und daß, während die Urnieren von *Paludina* vielleicht mit den Urnieren von *Lymnaeus* und *Planorbis* homolog sein könnten, sich von keiner Homologie mit den äußeren Excretionskörpern, »Urnierenzellen«, dieser Formen reden ließe.

Dies brachte die bedeutsame Tatsache ans Licht, daß es mehr als eine Art von »Urnieren« gibt, von denen die eine teils mesodermalen, teils ectodermalen Ursprungs ist (*Paludina*, *Planorbis*) und sich durch eine Pore nach außen öffnet; die andre aus Ansammlungen modifizierter Ectodermzellen, welche an der postoralen Reihe des Velum (*Lymnaeus*, *Planorbis*) oder, wie die späteren Untersuchungen von McMurrich (85) und Conklin (97) gezeigt haben, hinter dem Velum liegen können, besteht. Die zweifache Verwirrung, eine Folge des Versuchs, die Außennieren der Prosobranchier mit den Urnieren der Pulmonaten, und der entgegengesetzte Vorschlag, die Außennieren der Pulmonaten mit den »Ur-« oder »Kopfnieren« der Prosobranchier für homolog zu erklären, war wahrscheinlich stark durch den losen Gebrauch des Wortes »Urniere« unterstützt. In Verbindung hiermit ist es interessant, festzustellen, daß Korschelt und Heider (93), als sie Bobretzkys Zeichnungen für ihr Lehrbuch kopierten, die Erklärungen zu diesen so veränderten, daß »Ur« durch »Ex« ersetzt wurde.

Rabl (79) kam zum Schlusse, daß die Außennieren der *Planorbis* ein Teil des Velum seien, und daß sie nichts mit »Kopfnieren« zu tun hätten. Sarasin (82) beschrieb eine sehr intime Beziehung zwischen Velum und Außennieren bei *Bithynia tentaculata*. Wirklich beschreibt er die beiden unter einem Namen, die »Ansae«. Indem er über ihre Homologien spricht, sagt er: »Nach den Erfahrungen von Bobretzky, Bütschli und mir liegt auf jeder Seite der Prosobranchierembryonen ein Häufchen großer Ectodermzellen, das bei *Paludina* und *Bithynia* mit Wimperöffnung nach außen mündet. Nach Bütschli und Fol finden sich dieselben bei *Planorbis*. Ist dies richtig, so haben die Süßwasserpulmonaten zwei Organpaare, die als Urnieren zu deuten sind, ein vorderes und ein hinteres Paar. Hat Rabl recht, daß die von Bütschli zuerst gefundenen großen Zellen bei *Planorbis* und *Limnaeus* zum Velum gehören, so sind wahrscheinlich die von Bütschli und mir bei *Paludina* und *Bithynia* gefundenen Organe den hinteren Urnieren der Süßwasserpulmonaten homolog.«

Dieser letztere Standpunkt war zur Zeit, als McMurrich (85) schrieb, zweifellos der richtige. McMurrich kam zum Schluß, daß die »primitiven Ausscheidungsorgane« von *Fasciolaria* und *Fulgur* Teile des Velum sind. Er fand, daß sie bei *Fulgur*, sobald der Rand des Velum zuerst bemerkbar wird, erscheinen, er fand aber nicht die entsprechenden frühesten Stadien der Organe bei *Fasciolaria*. McMurrich glaubte, daß *Paludina* und *Bithynia*, bei denen sowohl Außennieren als auch Urnieren gefunden werden, den Schlüssel zu den Beziehungen dieser Organe geben. So sagt er (l. c. S. 438): »Es scheint wahrscheinlich, daß die Excretionszellen (Außennieren) ursprünglich Teile des präoralen bewimperten Randes des Velum waren, und, als deren excretorische Funktionen aus irgend einem Grunde mehr und mehr wichtig wurden, wurden sie vom Velum abgetrennt, und traten in einen höheren Grad der Entwicklung ein, so daß sie schließlich die primitiven ‚Kopfnieren‘ (Urnieren) ersetzten.« Nach dieser Ansicht sind die Zustände bei *Paludina* und *Bithynia* primitiv; dasselbe könnte man auch über die bei den Pulmonaten sagen. Außerdem schließt diese Ansicht ein, daß solche Prosobranchier, wie *Fulgur*, *Fasciolaria* und *Crepidula*, früher »Kopfnieren« hatten, daß diese aber durch »Excretionszellen« (Außennieren) ersetzt wurden. Es existieren gute Gründe für die Annahme, daß *Paludina*, *Bithynia* und die Pulmonaten nicht primitiv sind, und es liegt kein Beweis vor, daß die marinen Prosobranchier einst »Kopfnieren« besaßen. Angesichts dieser Tatsachen muß McMurrichs Theorie des Ersatzes fallen.

Erlanger (92), obgleich er die excretorische Funktion der »Ansae« von *Bithynia* leugnet, gibt zu, daß aus morphologischen Gründen diese Zellen als Äquivalente der sonderbaren Zellen des Velum der Pulmonaten und der marinen Prosobranchier angesehen werden könnten.

Heymons (93) führte eine andre Quelle der Verwirrung ein, indem er die Homologie der Excretionszellen der Opisthobranchier mit den Außennieren der Prosobranchier nachzuweisen suchte. Diese Homologie erscheint weit hergeholt, da die Excretionszellen von *Umbrella* nahe dem Anus der Larven gelegen sind, obgleich sie ihren Ursprung viel weiter nach vorn haben. Dieser Unterschied in der Lage schien Heymons nicht von Wichtigkeit, da, wie er sagt, McMurrich schon nachgewiesen habe, daß die Excretionszellen in verschiedenen Abständen hinter dem Velum liegen können. Conklin (97) antwortet hierauf: »Dieser Unterschied in Lage aber scheint mir ein sehr bedeutender zu sein. In allen Prosobranchiern liegen diese Zellen direkt hinter dem Velum, während in *Umbrella* sie von dieser Struktur durch beinahe den ganzen Durchmesser des Embryo getrennt sind. Weiter würde die Tatsache, daß sie in das Innere der *Umbrella* sinken, bedeuten, daß sie von den Excretionszellen der Prosobranchier verschieden sind.«

Es ist ganz augenscheinlich, daß in der so weit berücksichtigten Literatur von wenigstens drei Arten von »Urnieren« gesprochen wurde, von denen zwei öfters an demselben Tier vorkommen. Um die Unterschiede klar hervorzuheben, scheint es mir gerechtfertigt, die teilweise mesodermalen, teilweise ectodermalen Strukturen der Prosobranchier und Pulmonaten, die aus der inneren Körperhöhlung durch eine Pore nach außen münden, als Urnieren zu bezeichnen; die modifizierten Ectodermzellen, die bei den Süßwasserpulmonaten und den Süßwasser- und marinen Prosobranchiern auch bei Gegenwart der Urnieren vorkommen mögen, aber als Außennieren zu bezeichnen, und schließlich solche Organe, wie sie HEYMONS bei *Umbrella* vorfand, einfach Excretionszellen zu nennen.

Wer die schönen schematischen Bilder der mesodermalen Urniere, die uns nach STAUFFACHER (98[2]) die *Trochophora* von *Cyclas cornea* zeigt, gesehen hat, kann ähnliche Gebilde bei den Prosobranchiern und Pulmonaten nie mit den Außennieren verwechseln, oder auf eine Homologie zwischen den zwei Arten von »Urnieren« schließen. MEISENHEIMER hat aber (98[1]), indem er an *Limax maximus* die Urniere vom frühesten bis zum letzten Stadium verfolgte, Gründe vorgeführt, die einen zwingen, diesem Organ einen rein ectodermalen Ursprung zuzuschreiben. Die Gründe, die STAUFFACHER bei *Cyclas* vorführt, sind überzeugend, daß bei diesem Lamellibranchier die Urniere hauptsächlich mesodermalen Ursprungs ist. Es scheint daher nötig, wenigstens vorläufig, noch eine vierte Art »Urniere« anzunehmen, und die vorgeschlagene Klasse der wirklichen Urnieren je nach dem Ursprung ihrer für den Excretionsprozeß selbst wichtigen Teile, in mesodermale und ectodermale Urnieren einzuteilen.

Es ist von Wichtigkeit, daß die Urnieren andrer Lamellibranchier, Pulmonaten und Prosobranchier untersucht werden, um festzustellen, ob die Unterschiede, auf die STAUFFACHERS und MEISENHEIMERS Arbeiten hindeuten, allgemein sind, oder ob trotz aller Vorsicht doch noch Täuschungen vorliegen. Jedenfalls kann im jetzigen Stadium unsres Wissens keine Homologie zwischen den Urnieren der Prosobranchier, Pulmonaten und Lamellibranchier festgestellt werden, höchstens wäre es unter den zweifellos mesodermalen und unter den zweifellos ectodermalen Organen dieser Tiere tunlich.

Jedenfalls ist zwischen den mesodermalen Urnieren und den ectodermalen Außennieren, wie sie bei *Fasciolaria*, *Fulgur*, *Crepidula* u. a. vorkommen, keine Homologie festzustellen. Wie verhalten sich aber die ectodermalen Urnieren zu den Außennieren? Ich glaube, daß man auch hier nicht von einer Homologie reden darf, denn was die eigentliche Struktur dieser Organe und deren Entwicklung angeht, so sind die Unterschiede zwischen ectodermaler Außenniere und ectodermaler Urniere ebensogroß als die zwischen Außenniere und mesodermaler Urniere. Daß im ersteren Fall die zwei Arten zufällig aus demselben Keimblatt entstehen, sollte kein Grund sein, sie als homolog anzusehen. Die Logik, die dies benötigt, muß schließlich die drei Keimblätter und alle organischen Gebilde, seien sie auch der verschiedensten Art, homologisieren!

Conklin (97) fand, daß die »äußeren Excretionszellen« (Außennieren) von *Crepidula* keine Verbindung mit dem Velum haben. Dies ist auch in historischem Sinne von *Fasciolaria* wahr, denn hier treten diese Organe lange vor dem Velum auf, so daß deren endliche Verbindung mit diesem keine primitive ist, wie McMurrich uns glauben machen wollte, sondern sekundär, und durch ihren Ursprung unmittelbar unter dem Entstehungsort des Velum veranlaßt. Die Außennieren von *Fasciolaria* sind daher grundsätzlich ebensowenig wie die von *Crepidula* Teile des Velum.

Ich möchte aber nicht den Eindruck erwecken, mich auf die Seite derer geschlagen zu haben, die behaupten, daß eine Zelle nicht gleichzeitig velar und excretorisch sein könne. Bei *Fasciolaria* sind einige der accessorischen Außennieren sicher Teile des Velum. Während es mir wichtig scheint, daß die weithergeholten Vergleiche zwischen »Ur-« oder »Kopfnieren«, Außennieren und Excretionszellen (*Umbrella*), und die Verwirrung, die diese Vergleiche zur Folge gehabt haben, in ihrem wahren Lichte verstanden werden, und durch wirkliche Beziehungen ersetzt, bin ich nicht der Ansicht, daß die Homologien zwischen Außennieren der Prosobranchier und Pulmonaten zu streng gehalten werden sollten. Unzweifelhaft existiert eine allgemeine Verwandtschaft zwischen den Außennieren dieser zwei Ordnungen von Gastropoden, aber das Vorkommen dieser Art Ausscheidungsorgan an verschiedenen Regionen des Ectoderm derselben Larve zeigt, daß fein ausgedachte Homologien nicht viel bedeuten mögen, denn die Wichtigkeit der Außennieren ist hauptsächlich physiologisch, und ihre Tätigkeit kann, wenn nötig, von andern Ectodermzellen ergänzt oder übernommen werden.

Baltimore, 15. Dezember 1904.

Nachtrag.

Kurz nach Absendung meines Manuskriptes erschien eine Arbeit, »Amitosis in the Embryo of *Fasciolaria*«, American Naturalist, Vol. XXXVIII, von H. L. Osborn, die ich hier noch besprechen will. Osborn, indem er über die Struktur der Kannibalen schreibt, sagt

(l. c. S. 871) »there is a throat and a small amount of ectoderm, not nearly enough to enclose the yolk spherules (Eier)«; und S. 877, »The gastrulas before they have swallowed the food ova are (according to information and drawings access to which I owe to the kindness of Prof. McMurrich) very queer-looking objects on account of the very ample folds of the ectoderm to allow for the distension which is to follow. One would expect that the entoderm would be equally so, in order to receive the ova into an endoderm lined cavity, but after much study of this point, I am convinced that unusual as it is, there is not enough endoderm to enclose these ova, but only a very small amount reaching out a short distance from the throat in all directions ....«. »A study of various series agrees in showing only one very thin layer the ectoderm in contact with the ova except near the stomodaeum.«

Daß Osborn das ectodermale Häutchen der Kannibalen nicht fand, ist begreiflich, da diese Membran außerordentlich dünn ist, und in Schnitten nach den gewöhnlichen Methoden angefertigt, leicht zerstört wird. Ich hatte solche Schwierigkeiten, daß selbst nach Anwendung der Kreosotmethode meine besten Schnitte nicht immer tadellos waren. Trotzdem zeigten sie eine kontinuierliche ectodermale Membran, über deren Existenz die »very ample folds« der Pre-Kannibalen überhaupt keinen Zweifel lassen können.

Da das Entoderm nicht kontinuierlich und an vielen Stellen so dicht mit dem Ectoderm verschmolzen ist, daß man kaum die zwei Membranen unterscheiden kann, so folgt natürlich, daß die Abwesenheit des Ectoderm in Osborns Schnitten, die Abwesenheit des Entoderms zur Folge hatte. Osborn hat recht, wenn er sagt »there is not enough endoderm to enclose these ova«, aber nicht in dem Sinne, wie er es gemeint hat, denn die Schnitte, die gar kein Entoderm zeigten, konnten keine Vorstellung von dem unterbrochenen Zustande dieser Membran geben.

In bezug auf die Außennieren sagt er S. 874: »The physiological significance of this organ is indicated by its name. As usual with prosobranchs, the embryo develops inside an impervious capsule consequently it is of great importance that the wastes from its actively developing tissues be kept out of contact with them. This is done by intra-cellular storage. The barrier of living cytoplasm at the outer end of the cell keeps the cell the better from disintegrating and the contained material from escaping. This device reminds one of the Lepidoptera and their scales as reservoirs for waste nitrogen during metamorphosis«. »Amitosis here is clearly in accordance with the view, that senescence and amitosis are closely related; for these cells are plainly reaching the end of their career. Though they are for the time a part of a very young embryo, the organ is no more an integral part of the embryo, than the embryonic membranes of Arachnids and insects, in which amitosis is well known to be associated with senescence. It is really merely an embryonic structure. It is not however clear, that the active secretory function of these cells is a cause of the amitosis.«

Daß die Außennieren als Sammelplätze von Abfallprodukten funktionieren, ist zweifellos wahr, da sie zur Zeit ihrer Abstoßung noch von bedeutender Größe sind. Die Verkleinerungen, die mit zunehmendem Alter an ihnen bemerkbar sind, zeigen aber schon für sich, daß die Außennieren nicht nur als Sammelplätze zu deuten sind. Die nuclearen Aktivitäten, die ich im vorhergehenden beschrieben habe, zeigen, daß die angesammelten Stoffe in eine andre, meines Erachtens lösliche, Form umgearbeitet werden. Daß solche Excretion Nachteile für die in einer undurchlässigen Kapsel enthaltenen Embryonen mit sich führen würde, ist ganz klar, aber die Kapseln sind nicht, wie Osborn annimmt, undurchlässig, wovon man sich leicht durch sehr einfache Experimente überzeugen kann; z. B. trocknen die dem Wasser entnommenen Kapseln in einem Tage ganz aus, und wenn man sie in einer zu geringen Menge für mehrere Tage aufbewahrt, kann man den schlechten Einfluß, den Störung des normalen Gas- und Flüssigkeitswechsels mit sich führt, leicht an den Embryonen erkennen.

Die Aktivität der amitotisch entstandenen und sich teilenden Kerne, von denen Osborn überhaupt nicht spricht, kann nicht als Zeichen der Senescenz gedeutet werden, sondern ist mit hohem Stoffwechsel verknüpft. Aus diesem Grunde allein ist die Ansicht, sie seien nicht integrale Teile des Embryo, falsch, aber selbst, wenn sie nur als Sammelplätze von stickstoffhaltigen Abfallprodukten funktionierten, so wären sie insofern, als diese Funktion von Wichtigkeit im Leben der Larven ist, doch integrale Teile der Embryonen, die nicht ohne diese oder gleichwertige Organe bestehen könnten.

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Erklärung der Abbildungen.

Abkürzungen:

Cyl, Zylinder;
Dt, Dotter;
Ect, Ectoderm;
End, Entoderm;
End.Ex, entodermale Excretzellen;
ExK, Excretkörper;
ExK', sekundäre Excretkörper;
Fs, Fuß;
Ftstz, Fortsatz;
gr.Rg, granulärer Ring;
Hb, Haube;
Ht, Häutchen;
Hin, hinteres Ende;
hin.Bl, hinteres Bläschen;
Kpf.bl, Kopfbläschen;
Mak, Makromeren;
Md, Mundöffnung;
Mes, Mesoderm;
op.Gr, optische Grube;
Pfrpf, Pfropfen;
Syn, Syncytium;
Vac, Vacuole mit klarer Zone verbunden;
Vac', Vacuole lose im Kern;
Vac'', Vacuole nach außen entschlüpfend;
Verb.Rg, Verbindungsring;
Vel, Velum;
Vor, vorderes Ende.

Tafel VI—IX.

Alle Bilder, bis auf eins, wurden mittels des Abbeschen Zeichenapparates verfertigt, selbst die zusammengesetzten, wie Fig. 2 und 12, deren Teile jeder soweit wie möglich erst separat abgezeichnet wurde. Alle Zeichnungen wurden zur Ebene des Tisches gemacht. Sämtliche Figuren sind hier auf die Hälfte der Originale reduziert. Die Vergrößerungsangaben beziehen sich auf diese reduzierten Bilder.

Fig. 1. Pre-Kanniballarve. Vorderes Ende nach oben, wo sich die Kopfblase mit ihrem schäumigen Ectoderm klar wiedergibt. Vergrößerung D Obj. X 4, Oc. 2. Zeiss.

Fig. 2. Pre-Kanniballarve. Aus Dünnschnitten rekonstruierte Figur. Vorderes Ende nach oben; Zylinder etwas oberhalb der Vertikalmittellinie. Hinteres Ende sehr unregelmäßig. Vergrößerung 7 Obj. X 1, Oc. 2. Leitz.

Fig. 3. Querschnitt durch eine Larve desselben Alters, wie in Fig. 2, in der Ebene des Zylinders. Mitten ist der Dotter, nach außen passierend ist Entoderm, Mesoderm und Ectoderm. Oben rechts und links am Zylinder sind die ersten Anlagen der Excretkörper oder Außennieren. Vergrößerung 12 Obj. X 1, Oc. 2. Leitz.

Fig. 4. Ein horizontaler Schnitt durch die Larve, die in Fig. 2 aus Dünnschnitten rekonstruiert ist. Vergrößerung 7 Obj. X 1, Oc. 2. Leitz.

Fig. 5. Horizontalschnitt durch den Zylinder der Larve Fig. 2. Unterer Teil stark bewimpert und vacuolisiert, eine Degeneration, die dem Durchbruch des Mundes zuvorgeht. Rechts und links die Excretkörper. Erste Anzeichen der Amitose. Vergrößerung 12 Obj. X 3, Oc. 2. Leitz.

Fig. 6. Junge Kanniballarve kurze Zeit nach Durchbruch des Mundes. Die Larve liegt auf ihrer linken Seite; vorderes Ende nach oben. Der rechte Excretkörper zeigt sich deutlich; unter ihm der Mund, hinter ihm vier Dotterkugeln, die von den Makromeren der Furchungsperiode herstammen, die aber erst jetzt, wo die Larve durch Aufnahme von Eiweißsubstanz etwas geschwollen ist, sich zeigen. Vergrößerung 3 Obj. X 3, Oc. 2. Leitz.

Fig. 7. Kannibal, der zwei Eier verschluckt hat, von der ventralen Seite gesehen. Der Mund liegt zwischen den zwei Excretkörpern; unter dem rechten stehen zwei Makromeren heraus. Im Vergleich mit früheren Stadien ist diese Larve viel regelmäßiger. Vergrößerung D Obj. X 2, Oc. 2. Zeiss.

Fig. 8. Kannibal, der schon vier Eier verschluckt hat und ein fünftes bewältigt. Auffallend sind die großen Außennieren, die jedoch nicht ein älteres, sondern ein jüngeres Stadium als Fig. 7 darstellen. Größe, Anzahl der verschluckten Eier usw. lassen nicht auf Alter schließen, vielmehr der histologische Zustand der Außennieren. Vergrößerung D Obj. X 2, Oc. 2. Zeiss.

Fig. 9. Kannibal, der 14 Eier verschluckt hat. Außennieren in viel höherem Grade der Entwicklung als in vorhergehenden Bildern. Zellwände sind jetzt bleibend da und die Kerne haben schon in einigen Fällen amitotische Teilungen durchgemacht. Vergrößerung 40 Diam. Erect Image Dissecting Microscope. Leitz.

Fig. 10. Vollgemästeter Kannibal. Kopfbläschen nach oben. Solch eine Larve mag über 300 Eier enthalten. Vergrößerung 20 Diam. Erect Image Dissecting Microscope. Leitz.

Fig. 11. Querschnitt durch einen etwas älteren Kannibalen. Mesoderm zeigt sich nicht in dieser Ebene. Viele der Eier sind schon zerplatzt, in andern teilen sich die Keimbläschen durch Amitose. Vergrößerung 3 Obj. X 3, Oc. 2. Leitz.

Fig. 12. Die Ventralseite eines älteren, aus Schnittpräparaten zusammengestellten Kannibalen. Kopfbläschen, Außennieren, Mund und Fuß sind einem Individuum entnommen, der übrige Körper einem andern. Das Kopfbläschen ist größer als wie gewöhnlich, doch in andern Hinsichten ist es normal. Das Bild zeigt genau die an einem einzelnen Exemplar überhaupt nicht wahrzunehmenden Verhältnisse der äußeren Organe. Vergrößerung 3 Obj. X 3, Oc. 2. Leitz.

Fig. 13. Teil der Oberfläche eines Excretkörpers im Stadium der Fig. 8. Zellwände nicht wahrnehmbar. Vacuolen sehr verschiedener Größe kommen zu dieser Zeit vor. Die Kerne sind meistens in der Nähe der großen Vacuolen. Vergrößerung 12 Obj. X 3, Oc. 2 X 18 Tubenlänge. Leitz.

Fig. 14. Optischer Schnitt durch einen Excretkörper, etwas älter als in Fig. 13. Zellwände sind jetzt wahrnehmbar. Die Kerne scheinen durch die größeren Vacuolen zu passieren. Eine der Zellen hat gar keine sehr großen Vacuolen mehr, aber, eine Anzahl kleiner, mitten unter welchen sich der Kern befindet. Memorandum der Vergrößerung existiert nicht.

Fig. 15. Optischer Schnitt durch ein etwas älteres Stadium. Stark vergrößert. Die großen Vacuolen sind ganz verschwunden; die Kerne haben eine centrale Lage aufgenommen, und den Zellwänden entlang sind Ansammlungen von Körnchen. Memorandum der Vergrößerung existiert nicht.

Fig. 16. Querschnitt durch die Excretkörper einer Larve etwas jünger als die in Fig. 9. Oben sind zwei Außennieren durch die dünne Membran des Kopfbläschens verbunden, nach unten ist der Schnitt offen, weil das Kopfbläschen erst von der Larve hat entfernt werden müssen. Vergrößerung 3 Obj. X 3, Oc. 2 X 20 Tubenlänge. LEITZ.

Fig. 17. Einige Zellen aus der Mitte eines Excretkörpers Fig. 16. Vergrößerung 12 Obj. X 1, Oc. 2. LEITZ.

Fig. 18. Einige Zellen aus der Region des Verbindungsringes Fig. 16. Vergrößerung 7 Obj. X 8, Oc. 2. LEITZ.

Fig. 19. Ältere Außenniere, von der dem Embryo zugewendeten Seite gesehen. Memorandum der Vergrößerung existiert nicht.

Fig. 20. Optischer Schnitt durch eine ältere Außenniere, in der Amitose sich abspielt. Die Zellen sind jetzt Polygone, nahe zusammengedrängt, und von kleineren Vacuolen erfüllt. Vergrößerung A Obj. X 4, Oc. 2. ZEISS.

Fig. 21. Querschnitt durch eine Außenniere, etwas älter als Fig. 19, an welch letzterer die Ebene des Schnittes angegeben ist. Der Hohlraum ist die Höhle des Näpfchens; unten rechts und links zeigen sich Schnitte durch den Verbindungsring. Vgl. Fig. 19. Vergrößerung 3 Obj. X 3, Oc. 2 X 20 Tubenlänge. LEITZ.

Fig. 22. Älterer Schnitt in derselben Ebene. Zeigt, daß der Verbindungsring zerschmolzen ist und einen in den Hohlraum des Näpfchens hinein projektierenden Pfropfen gebildet hat. Unten rechts und links ist der Pfropfen mit dem Ectoderm der Larve verbunden. Vergrößerung 3 Obj. X 8, Oc. 2. LEITZ.

Fig. 23. Pfropfen aus Fig. 22. Vergrößerung 12 Obj. X 3, Oc. 2. LEITZ.

Fig. 24. Vier Pfropfenkerne. Vergrößerung 12 Obj. X 8, Oc. 2 X 20 Tubenlänge. LEITZ.

Fig. 25. Kopfende einer vollentwickelten Veliger-Larve. Das Kopfbläschen mit seinen zwei Tentakeln liegt mitten im Velarfeld. Zwischen der präoralen und der postoralen Reihe des Velums ist die Mundöffnung, und unter der postoralen Reihe zeigt sich deutlich der Fuß mit seiner Drüse. Unter dem Velum hängen rechts und links die Excretkörper, die nicht immer von gleicher Größe sind. Die numerierten Linien, die durch das Bild in verschiedenen Richtungen gezogen sind, geben die Ebenen, durch die ihnen gleich numerierte Schnitte geführt sind (vgl. Textfig. 4). Vergrößerung 20 Diam. Erect Image Dissecting Microscope. LEITZ.

Fig. 26. Querschnitt durch eine vollentwickelte Außenniere. Ebene des Schnittes an Fig. 25 angegeben. Vergrößerung A Obj. X 4, Oc. 2 X 20 Tubenlänge. ZEISS.

Fig. 27. Längsschnitt durch eine vollentwickelte Außenniere. Ebene des Schnittes an Fig. 25 angegeben. Dieser Schnitt zeigt Näheres über die Verhältnisse, die bei einer mehr als gewöhnlich innigen Verbindung zwischen Velum und Außennieren obwalten. Vergrößerung 3 Obj. X 3, Oc. 2. LEITZ.

Fig. 28—39. Optische Schnitte durch Außennierenzellen, in denen Amitosen sich abspielen. Vergrößerungen 12 Obj. X 3, Oc. 2. LEITZ.

Fig. 40—42. Dünnschnitte durch amitotisch entstandene Kerne der Außennierenzellen. Besonders bemerkenswert sind die unregelmäßigen klaren Zonen um die Nucleolen herum. Vergrößerung 12 Obj. X 6, Oc. 2 X 2. LEITZ.

Fig. 43. Eine isolierte Vacuole stark vergrößert, freier Hand gezeichnet.

Fig. 44. Eine einem vollentwickelten Excretkörper aus der Mitte entnommene

Excretzelle. Bemerkenswert ist die lange, gestreckte, prismatische Form und das haubenartige obere Ende der Zelle. Vergrößerung 12 Obj. X 3, Oc. 2 X 18 Tubenlänge. Leitz.

Fig. 45. Längsschnitt durch eine solche Zelle. Vergrößerung 12 Obj. X 3, Oc. 2. Leitz.

Fig. 46. Längsschnitt durch ein Velum, in dem einige Zellen der postoralen Reihe zu sekundären Excretzellen (*ExK*) verwandelt sind. Vergrößerung 3 Obj. X 3, Oc. 2 X 20 Tubenlänge. Leitz.

Fig. 47. Seitliche Oberfläche eines Velums; oben die prä- unten die postorale Reihe, letztere mit sekundären Excretzellen (*ExK'*). Vergrößerung 3 Obj. X 3, Oc. 2 X 20 Tubenlänge. Leitz.

Fig. 48. Kopfbläschen eines vollentwickelten Veligers, von oben gesehen. Rechts und links sind die Tentakel, und zwischen ihnen einige sekundäre Excretzellen. Vergrößerung 40 Diam. Erect Image Dissecting Microscope. Leitz.

Fig. 49. Eine aus mehreren durch einen älteren Kannibalen geführten Querschnitten zusammengesetzte Figur. Vergrößerung 3 Obj. X 3, Oc. 2. Leitz.

Fig. 50, 51, 52. Dünnschnitte durch Zellen des Schlundes der in Fig. 49 erwähnten Larve. Fig. 50 u. 51 sind durch Zellen der seitlichen und hinteren Wandung des Schlundes; Fig. 52 der vorderen, wo die Verhältnisse verschieden sind. Vergrößerungen 12 Obj. X 3, Oc. 2. Leitz.

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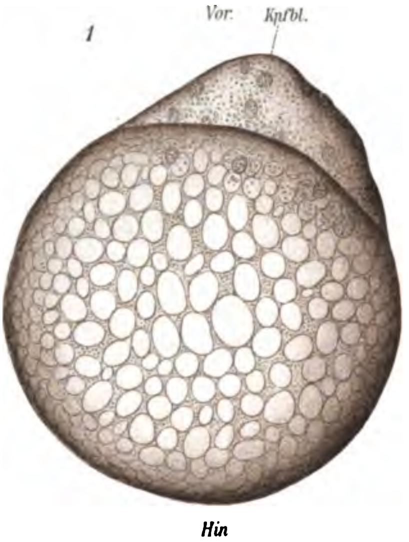

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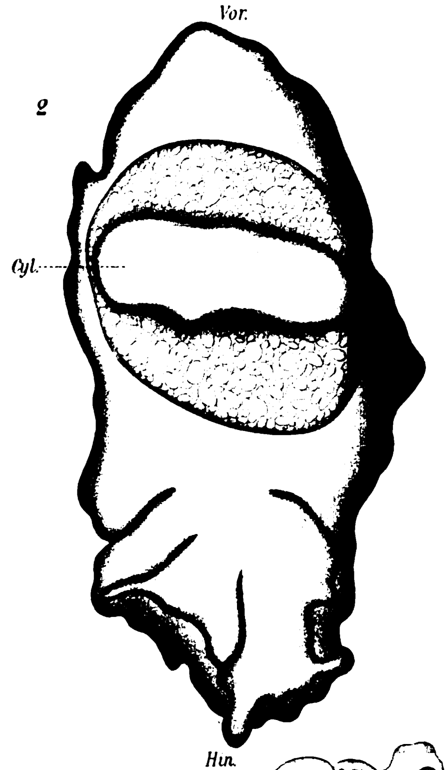

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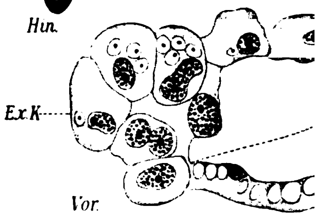

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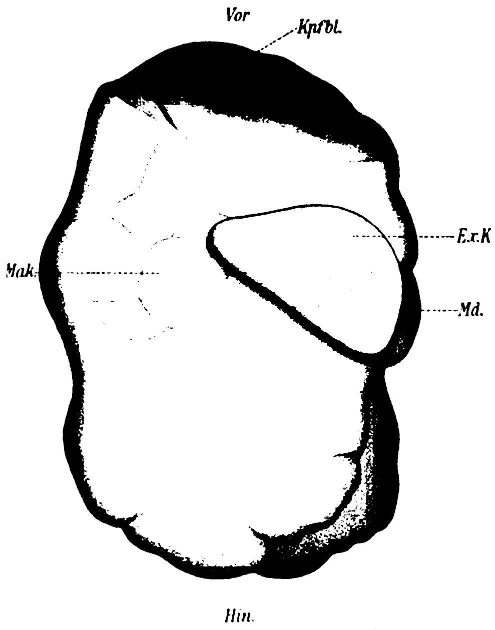

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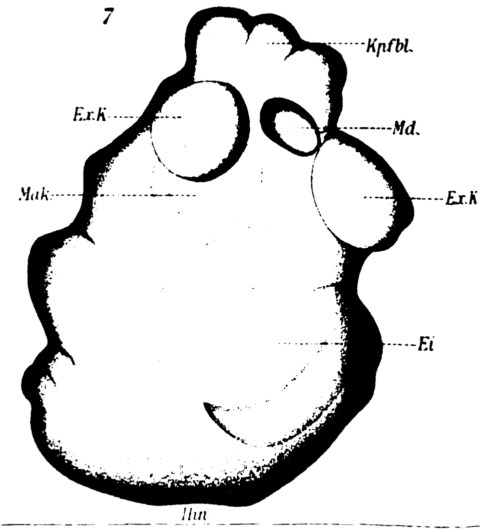

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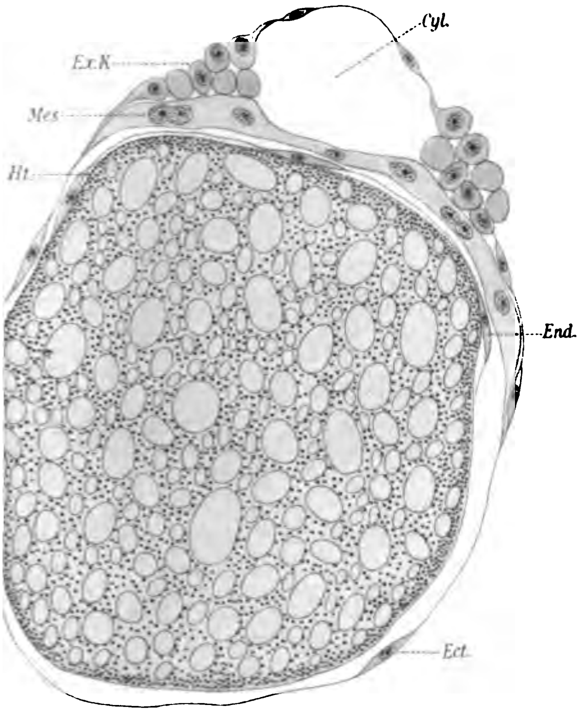

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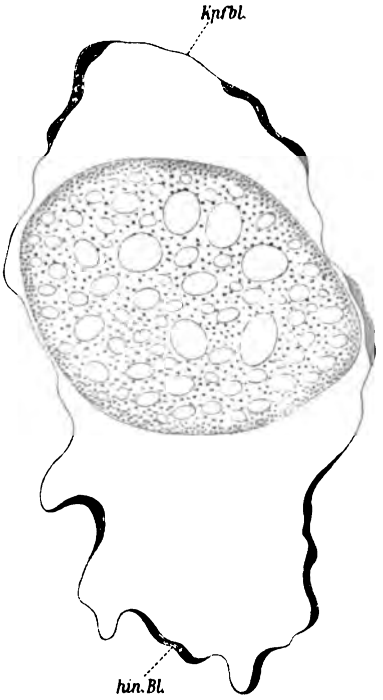

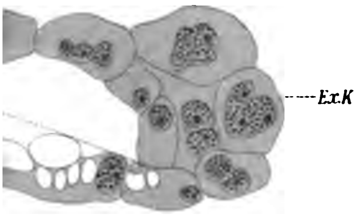

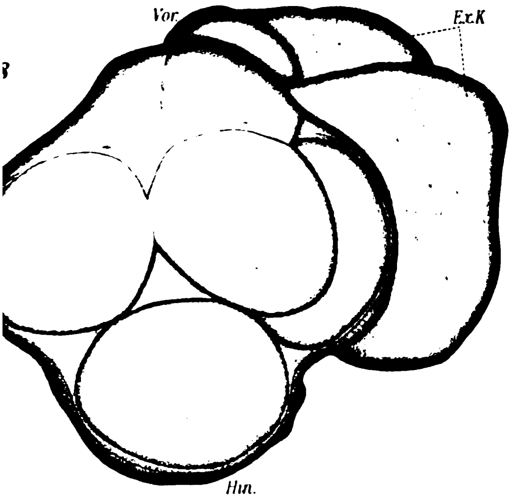

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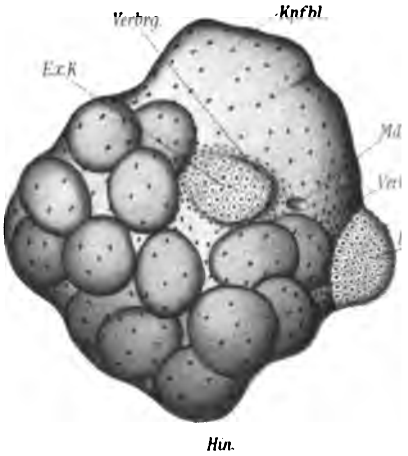

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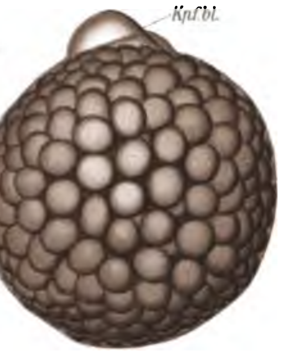

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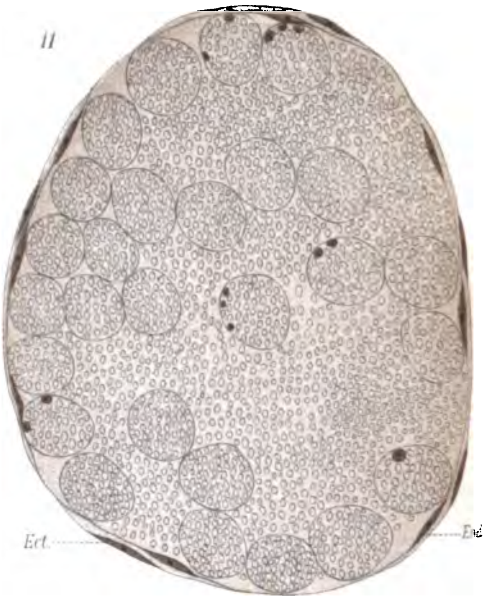

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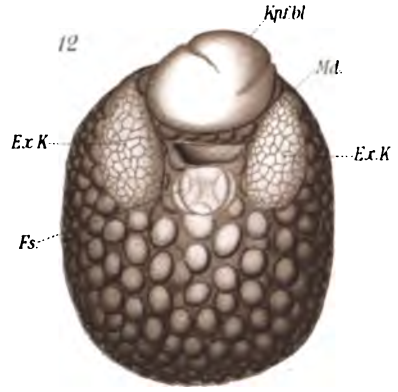

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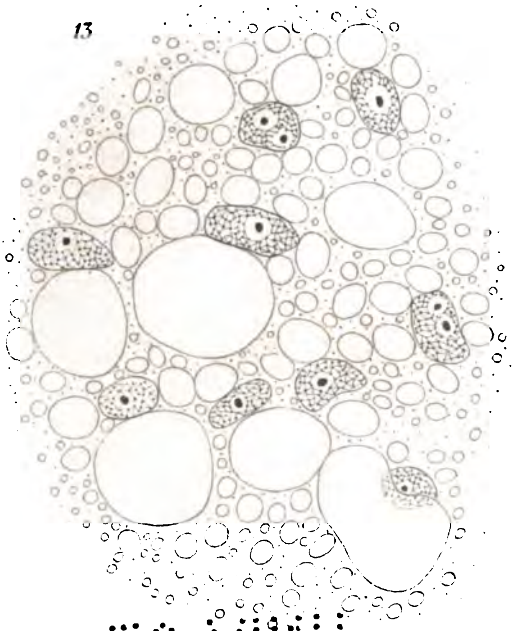

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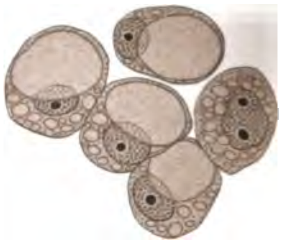

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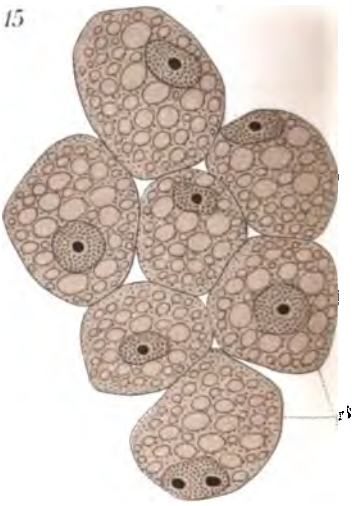

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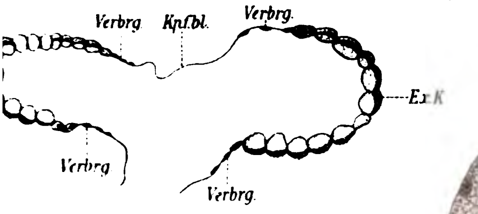

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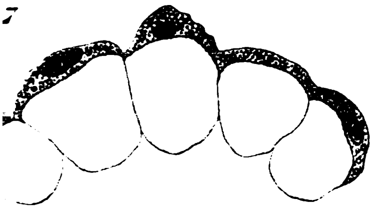

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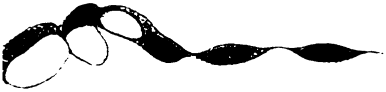

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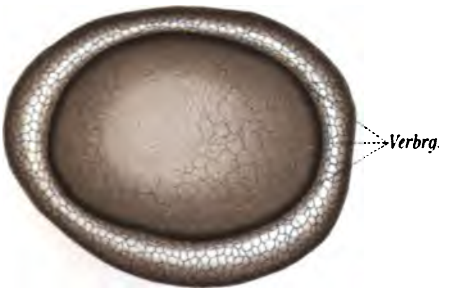

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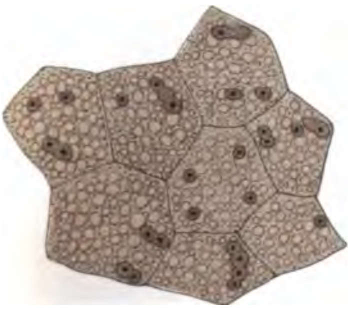

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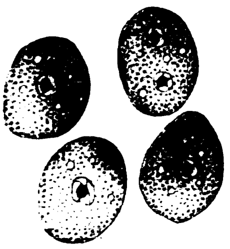

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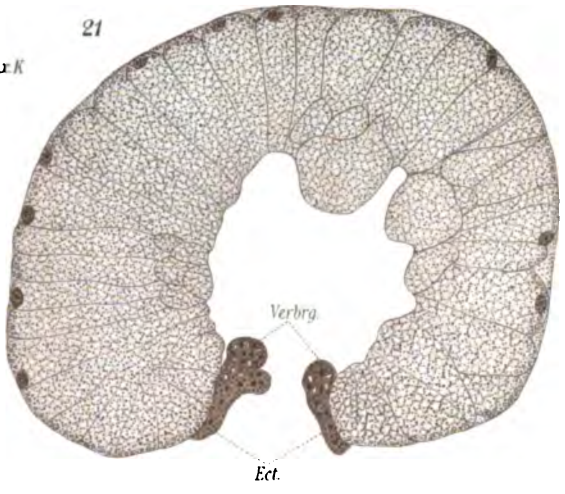

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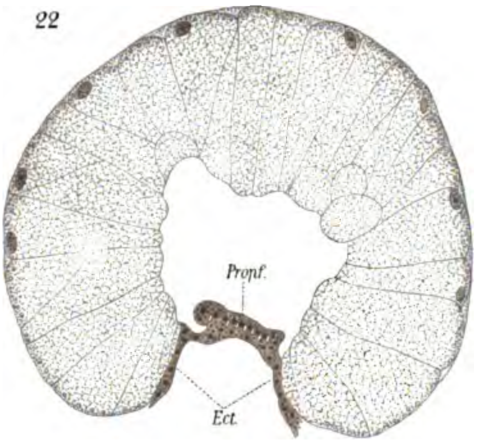

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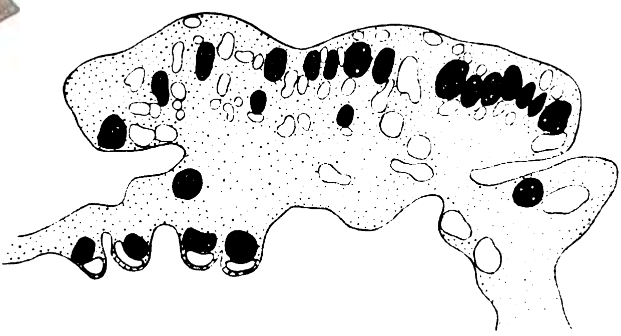

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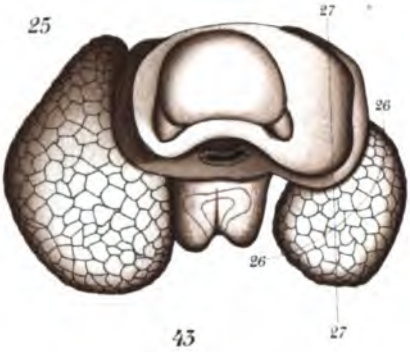

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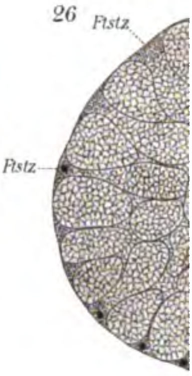

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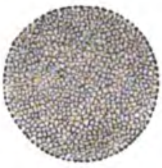

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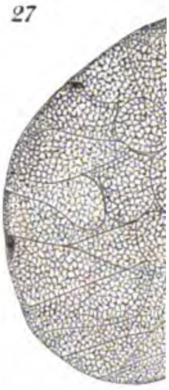

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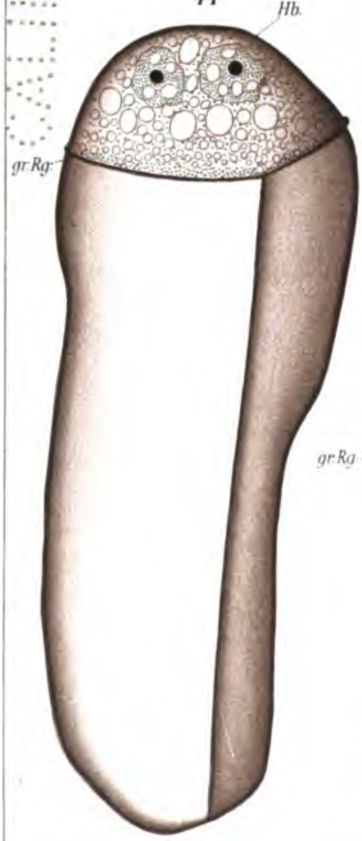

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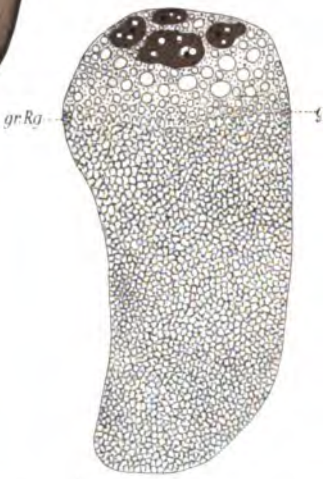

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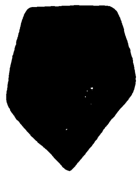

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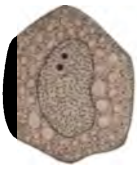

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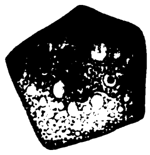

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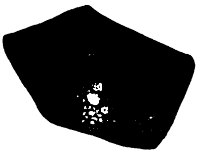

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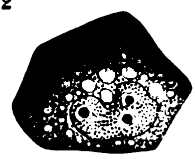

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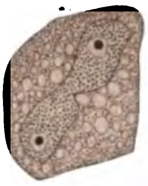

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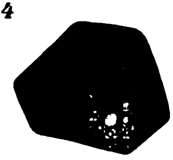

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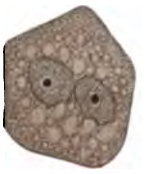

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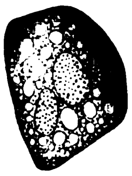

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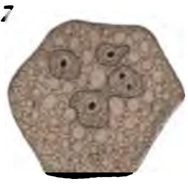

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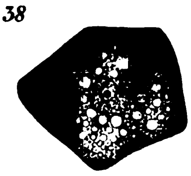

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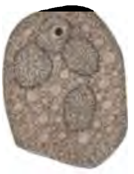

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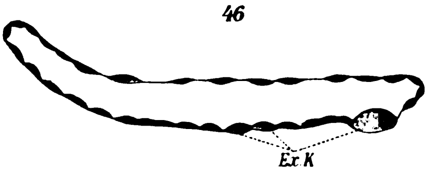

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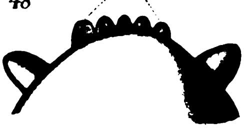

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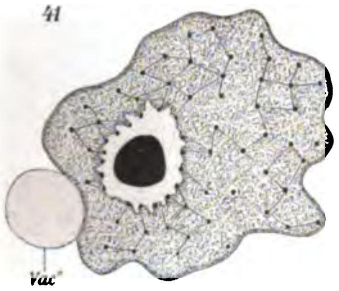

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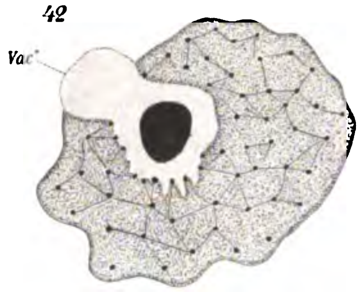

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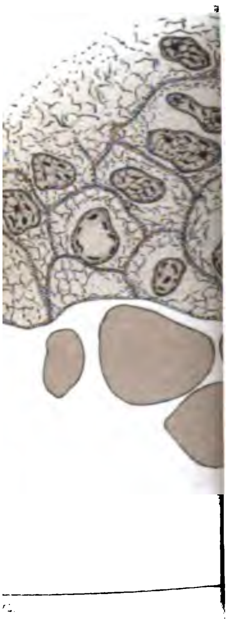

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BIOGRAPHICAL NOTE.

Otto Charles Glaser was born in Wiesbaden, Germany, on October 13th, 1880. His early education was secured in the private and public schools of Baltimore. In 1897 he entered the Johns Hopkins University as an undergraduate student. In June, 1900, he received the degree of Bachelor of Arts. Since October, 1900, he has been a graduate student in the Biological Department of the Johns Hopkins University.

OVEMENT AND PROBLEM SOLVING IN OPHIURA BREVISPINA

By

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MOVEMENT AND PROBLEM SOLVING IN OPHIURA BREVISPINA[1]

BY

O. C. GLASER

WITH FIVE FIGURES

INTRODUCTION

The observations and experiments which I shall describe and discuss in the following pages were made in the Marine Biological Laboratory at Wood's Hole, for the purpose of testing Preyer's conclusion that ophiurans are intelligent animals. In spite of the fact that there is still much difference of opinion as to what we mean by intelligence, all will agree, I think, that it involves at least the ability to learn and to modify behavior in accordance with experience. Jennings ('06, p. 291) has formulated in the law of the resolution of physiological states, the way in which behavior is modified in experience: "The resolution of one physiological state into another becomes easier and more rapid after it has taken place a number of times." I have attacked the problem of intelligence in Ophiura brevispina from the point of view afforded by this law of resolution.

PROGRESSION

Progression in ophiurans has been described by a number of observers, including Romanes ('85), Preyer ('86), von Uexküll ('05) and Grave ('00). These writers agree as regards the general method of locomotion in ophiurans, but they have not described all of the movements which these animals perform. All of these authors have noticed two types of progression, the first of which may be visualized by the aid of Fig. 1, in which the arms are numbered, and so distinguished by heaviness of line, that the most active is the widest, the least active the narrowest.

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[1] Contributions from the Zoölogical Laboratory, University of Michigan, No. 107.

In movements conforming to type *I*, Fig. 1, *A*, the two arms *1* and *3* are used as a pair, whose strong backward stroke drives the animal in the direction indicated by the arrow. Arm *2*, which projects forward rather stiffly, serves only the function of guiding, this being also the effect of *4* and *5*, which are dragged behind. A slight modification of type *I*, *A*, is found in type *I*, *B*, in which the distal end of arm *2* waves from side to side, and in this manner adds to the propelling force furnished by *1* and *3*. Type *I*, *C*, is a further modification of *I*, *A*, in which arm *2* instead of bending only distally makes a stroke as effective as either that of *1* or *3*, and bends either to the right or to the left, so that the animal is propelled by two arms on one side and one on the other. The course is zigzag if regular alternations in the direction of the stroke of arm *2* occur but if this always falls on the same side the course is circular.

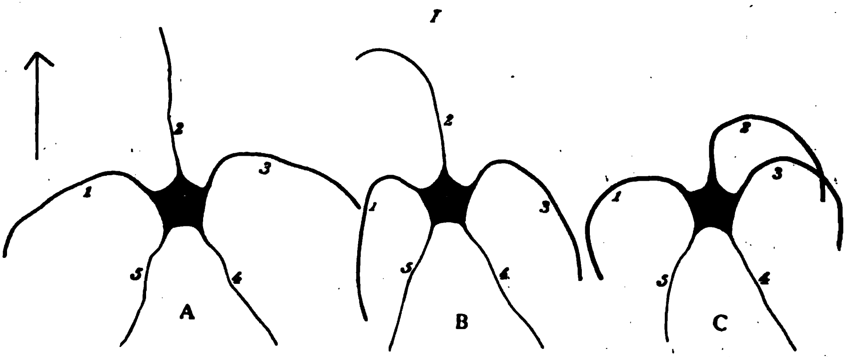

Fig. 1

The movements that fall within this type are variable to an extent which has not been pointed out. *I*, *A*, represents in its pure forms one of the two types which all previous writers have noticed, though Grave ('00) has also observed the modification *B*, of which *C* is the extreme case. Von Uexküll ('05), who calls this type of movement Typus Unpaar voran, says: "Beim Bewegungstypus Unpaar voran, zeigt sich welch grosse Unterschied in der Bewegungsamplitude des ersten und zweiten Gangpaares besteht. Letzteres verhält sich beinahe passiv. Doch kann es gelegentlich auch stärker in Aktion treten." Both Preyer ('86) and Grave ('00) state that the "posterior" pair is dragged behind, and I have never observed more than insignificant movements in it.

Type *II* (Fig. 2), observed by all of the writers mentioned, and called Typus Unpaar hinten by von Uexküll, may be described as two pairs of arms working synchronously, or alternately, the anterior pair initiating movement at one time, the posterior at another, or the movement may be begun by arms *2* and *4*; by *1* and *3*; by *2* and *1*; or by *3* and *4*; the only constant factor is the behavior of arm *5* which is invariably dragged behind.

A third type of movement, Fig. 2, *III*, not previously recorded, involves the activity of all the arms in such a manner that the animal is forced forward by three arms on one side and a pair on the other. This type may be thought of as a modification of *I*, *C*, in which arms *4* and *5* have become active, or as *II*, in which arm *5* has become active. Type *III*, is really *I*, *C*, plus an additional pair, and as in *I*, *C*, the course is zigzag if arm *2* alternates regularly from side to side, circular if the stroke falls always in the same direction.

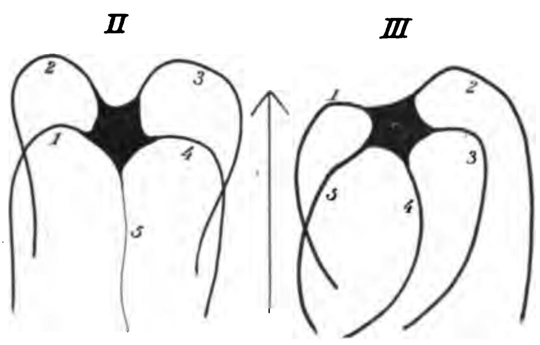

Fig. 2

It is not necessary to describe the finer variations to which these types of movements are subject; to point out, as has been done in von Uexküll's excellent paper ('05), how one may pass over into another, or how the course is affected by differences either constant or variable in the rate and strength of stroke of particular arms or particular combinations of arms. With the exception of type

Unpaar voran, in which according to von Uexküll effective movements occur in the two arms which are usually dragged passively behind, I have observed that Ophiura brevispina moves in practically all the ways in which it is possible for a pentaradiate animal of its construction to move.

INDIVIDUALITY

The movements described are directly dependent upon the pentaradiate symmetry, but this symmetry does not exhaust the possibilities of behavior. A little observation shows that each animal is unique at any given time and that while its movements fall within the system of classification proposed, they have peculiarities that distinguish them from other movements of the same type.

In general the movements may be either rapid or slow, and certain individuals seem on first acquaintance to be distinctly active or distinctly sluggish. More careful study shows, however, that very sudden changes of behavior occur, and that an active, rapidly moving animal may unexpectedly enter into a state of sluggishness that sometimes lasts for hours. I do not understand these sudden changes. They are not due to the conditions in the aquaria; they occur with great suddenness and not in all of the animals; they are not due to either gentleness or roughness in handling because either may or may not be followed by a change in the behavior of the same individual in successive trials. Possibly any sort of handling may, in certain physiological states, cause a change of behavior, but what the physiological state in which this occurs is, is hard to ascertain. In certain experiments in which I encumbered the arms with rubber tubes, after the manner of Preyer ('86), I frequently encountered the same sudden change from activity to passivity, and arms which were flexible and easily encumbered, would suddenly bend at their tips and stiffen, so that it was impossible to slip the tube over them. This stiffening might take place at the first trial, or some other one, and never again, or it might reoccur upon every attempt to encumber the arm.

Periodic changes from activity to sluggishness also occur.

Thus, in June of the present year, more than half of the animals I studied were very active and quickly responsive to stimuli during sometime of my acquaintance with them, but by August the whole race had changed. Perfectly fresh material brought into the laboratory in excellent condition and kept in large tanks of running sea-water, was so sluggish that I was forced to give up the experiments which I had planned for that month. None of the stimuli employed in June elicited reaction, and acids sufficiently concentrated to attack the skeleton, as well as the electrical current, resulted in nothing but a few spasmodic contractions with no attempt at progression or escape. What the reason for this change was is not certain. A sluggish individual almost always has very large bursal openings; in fact, it is possible to predict with considerable certainty the behavior of an individual by examining its ventral surface. The enlarged bursal openings may be consequences of the spawning process, and the periodic change of behavior of the breeding activities. O. brevispina begins breeding in June and ends in August. Late in June many individuals have spawned, and many have the enlarged bursal openings; by the middle of August all have spawned (Grave '00) and most of the individuals have the enlarged bursal openings. As the genital ducts lead into the bursæ—which in some species are used as brood-pouches—their enlargement may very well be due to sexual activity, which is a drain upon the animals, and undoubtedly leaves them in a state of physiological depression. If this view is correct, the enlarged bursal openings are the indices of a lethargic state following the breeding season.

Rapidity and sluggishness of movement have consequences of great importance in problem solving. Sluggish animals not only make fewer movements and take more time to perform them than active individuals, but they use in general fewer arms; their movements are less varied, and the arms very rarely come into contact with one another or cross. All this is very different with active individuals; their movements are quick and varied; they use relatively more arms, often move these through greater arcs than the sluggish animals; and, in addition, the arms touch and cross with great frequency. How "contacts" and "crosses" are related to activity and sluggishness is easy to see. An active individual using four arms in progression has a much greater opportunity to make "crosses" and "contacts" than if fewer arms were used. Very often when the animals move by means of two pairs of arms, the anterior pair is crossed by the posterior regularly. The same frequently happens when only three arms are used.

Contacts and crosses also depend on the length of the arms, as the chances that they will occur in long armed individuals are greater than in short. How important arm length is, is indicated in the following table in which are summarized observations on three individuals which were active, but differed in the lengths of their arms and also in the manner of using them. The effect of the latter factor emphasizes that of the former. The longest armed individual *A* used the "two pair of arms" stroke, only once in a total of 141 effective backward strokes, whereas the shorter armed individuals *B* and *C*, used this stroke eight times in 129 and four times in 126, respectively.

TABLE I

Individual	No. of Movements	Per cent Contacts	Per cent Crosses	No. of arms moved					
				1	2	3	4	5	
A	141	13	119	1	26	28	1	0	times moved
B	129	11	16	0	29	13	8	0	times moved
C	126	6	2	0	16	26	4	0	times moved

RIGHTING MOVEMENTS

Two types of righting movements were observed, only the first of which has been described by von Uexküll ('05) in an excellent paper illustrated by means of kinetoscope photographs, and by Grave ('00), who says: "Two adjacent arms straighten out so that together they form a straight line. On these arms as an axis the body revolves, being pushed over by the three remaining arms, but mostly by the median one of the three."

This description, correct as far as it goes, is incomplete. At the bases of the straightened arms, and in the interradial portion of the disc between them, movements occur whose effect is to bend the ventral surface in the direction indicated by the arrows. When this process, by which a small portion of the ventral surface is brought into the normal position (Fig. 3, *A*), has proceeded far enough, the animal is righted suddenly by its own weight, since while the process described has been going on, arms *3*, *4* and *5* have so elevated the dorsal surface of the disc that this falls into the normal position.

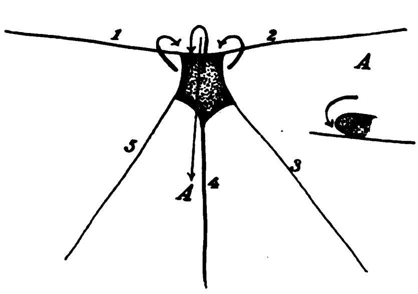

FIG. 3

In the second type of righting movement, Fig. 4, arm *2* curves near its base, and bends under the disc which, as in the previous case, is elevated by the other arms, particularly by *4* opposite *2*. The disc thus rotates on the base of *2* as a pivot, and after it has been sufficiently elevated, the animal falls into the righted position of its own weight.

The length of time required to execute the righting reaction was measured on eight individuals. I have summarized these results in Table II, in which are given the average time for each individual, as well as the maximum and minimum consumed. (See Table II.)

These averages of course do not show the differences between the successive individual rightings of any of the animals used. These differences were in some instances very large, and have had a great effect on the averages. (See Table III.)

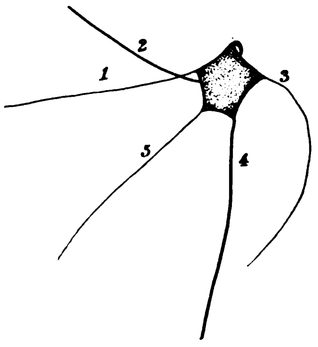

FIG. 4

These measurements show that the variations from the mean may be very great; that because an individual has righted itself very quickly a number of times is no reason for believing that it will continue to do so. in spite of those cases in which righting took place slowly, the

TABLE II

Average righting-times of eight animals

Date	Intervals	No. Trials	A	B	C	D	E	F	G	H
June 27	0	8	3.62″	3.62″	14.50″	4.12″				
27	5′	8	2.86	4.37	25.50	5.12				
27	3 hrs.	10	4.20	3.80	10.60	4.10	4.00″	16.20″	4.90″	3.60″
28	16 hrs.	10	5.10	5.60	6.40	43.30	5.10	10.30	5.10	4.40
30	64 hrs.	10		3.80	15.60	21.60	29.10	7.10	3.50	7.9
			Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min	Max. Min.	Max. Min.
			8″ 2″	14″ 2″	43″ 4″	198″ 2″	89″ 3″	39″ 3″	12″ 2″	24″ 2″

TABLE III

Individual righting-times of eight animals in many successful trials

A	B	C	D	E	F	G	H
3	5	7	2	4	8	5	3
4	3	5	5	3	12	5	4
3	3	12	4	3	9	6	3
2	2	7	3	4	17	4	3
5	5	20	6	4	12	5	2
3	3	12	4	3	17	4	3
3	5	34	4	5	13	3	3
7	3	19	5	4	39	2	7
3	4	19	2	4	22	3	3
3	2	31	3	6	13	12	5
3	7	8	6	4	9	4	4
3	5	28	4	3	5	10	4
3	4	43	8	5	3	4	4
3	4	33	4	4	15	6	3
3	4	14	4	6	7	6	8
2	5	28	10	4	4	4	4
4	3	10	3	4	14	4	4
3	4	8	4	7	11	5	3
4	3	23	3	7	24	4	5
3	3	6	3	7	11	4	5
3	4	10	3	4	6	3	5
3	4	12	5	29	4	2	7
8	4	8	3	31	3	4	5
7	5	17	4	35	7	3	3
3	4	7	4	12	8	3	24
4	4	5	9	7	6	6	6
3	3	4	29	37	10	3	5
4	4	6	23	38	3	2	6
7	7	5	15	89	15	4	9
7	4	5	20	9	9	5	9
5	4	4	29				
6	5	6	40				
7	14	6	18				
5	6	9	198				
3	6	5	7				
4	3	14	5				
	3	8	4				
	5	5	11				
	4	27	25				
	4	6	45				
	3	21	11				
	5	27	12				
	4	12	14				
	4	20	14				
	3	24	41				
	3	6	39				

records when averaged show that these animals, on the whole, may be expected to right themselves in less than 45 seconds. One fact of considerable interest is clearly demonstrated by the averages as well as by the individual records—there is no reduction in the amount of time required to perform the righting act; in other words, under normal conditions, these animals do not improve by practice in the execution of their righting movements.

PROBLEM SOLVING

The expression "problem solving" is almost self-explanatory. Under this heading, I have placed such behavior as an ophiuran exhibited when stimulated by interference more or less unusual, and from which it was able sooner or later to escape. What I did was to observe the way in which the escape was made—the problem solved—and how much time was consumed in doing it.

The problem—the same as that employed by Preyer—was to rid one or more arms of the small pieces of loosely fitting rubber tubing with which I encumbered them. In the selection of individuals for experiment, my choice was guided by two considerations: whether all the arms were approximately equal in length, neither broken, nor recently regenerated; and whether the individuals were not too active to make the observations easy to record.

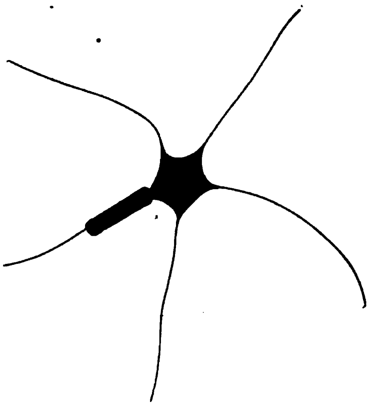
FIG. 5

When encumbered in the manner represented in Fig. 5, an ophiuran does many things, some of which are recorded in von Uexküll's photographs. At first it may pass through a brief latent period, during which it lies motionless on the bottom of the dish, and then it may crawl, dragging the encumbered arm behind it. Often the animal moves at an angle to the encumbered arm, or in rare cases in the direction of it. The

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progression may be of a very violent character involving many contacts and much crossing of arms, or the animal may simply writhe, without changing its location. If it does not move about, it usually waves one of its arms, especially the encumbered, in a horizontal plane, though the movements may also occur in a vertical plane and in circles. The encumbered arm is moved in a vertical plane oftener than the unencumbered ones; is frequently rubbed against the disc; against the adjacent arms; against the sides of the dish; and even against itself. Sometimes the encumbered arm is waved over the disc, much as a man waves a long whip, and then is "cracked," so that the encumbering tube moves nearer the distal end, and often slides off. When relieved the animal usually does not remain quiet, but continues its movements for a short time and makes several strokes that remove it from the place where the tube was gotten rid of. If at the instant of riddance the animal was not progressing, a short journey is begun at the moment of relief.

When encumbered on more than one arm, the latent period is longer than when only one arm is encumbered; the first movements are not through as great arcs, nor are they so long continued in any direction. One movement is succeeded rapidly, not by its duplicate, but by another in a different direction, and this by still another. The behavior changes constantly.

If all of the arms are encumbered, the above changes in behavior cease very soon, and an entirely different kind of action is begun. Instead of movements in the usual sweeping manner, the arms quiver and tremble. In one case, one arm (the first to be rid of its rubber tube) in particular attracted my attention by quivering when the rest of the animal was perfectly quiet. These quivering movements occur in a horizontal plane, and are so rapid, and many of them so slight, that it is impossible to record them accurately without special apparatus.

Of all these movements, several are more effective than many others in bringing about riddance. The most effective are the "whip movement," the "stripping movements;" certain of the "wavings," and violent progression which involves a number of different movements. Of these the whip movement is the rarest;

the violent progression next, whereas the strippings and the wavings are the commonest of all.

These observations open two ways in which the problem of resolution may be attacked; by studying the time taken to solve the problem and by noting the relative frequency of the most effective movements. The time and the frequency might both remain constant, or might change, or only one might change. As a reduction in the amount of time taken to solve the problem need not necessarily be due to an increase in the relative frequency of the most effective strokes, these two must be considered separately, although an increased frequency of strokes best fitted to solve the problem would involve a reduction in the amount of time. If a reduction in the amount of time required does occur, it means that the physiological state produced by the rubber tube has been resolved into the normal state more rapidly than it was resolved the first time. In other words, the animal has learned by experience.

The following Table IV contains my measurements of problem solving time. In every case the animal was given the same problem consecutively, viz: the same rubber tube was placed on the same arm, under the same conditions. As little time as possible was lost between trials.

TABLE IV*

Trials

Individual.......	1	2	3	4	5	6	7
A	3′ 45″	5′ 00″	2′ 00″	3′ 00″	6′ 00″		
B	0′ 30″	4′ 30″	1′ 00″	1′ 1′	4′ 00″		
C	0′ 45″	1′ 45″	0′ 1″	5″ 00″	1′ 30″		
D	2′ 00″	3′ 00″	7′ 30″	3′ 00″	3′ 00″	2′ 15″	3′ 30″
E	1′ 30″	0′ 45″	1′ 30″	1′ 40″	5′ 15″	3′ 30″	

*These measurements include the latent periods.

The number of trials recorded in Table IV is small. I was prevented from collecting more data by the sudden changes of behavior before alluded to. Other animals were tried but failed to react regularly even five times. The results as they stand, however, are worthy of confidence; they are representative of the whole behavior which is varied and uneven; like the measurements of righting time they neither increase nor decrease—the apparent increase being due to the failure to respond, for had this failure occurred sooner, some of the last measurements would have been smaller than the first. Fatigue played no part in the result, as the figures are too uneven.

The objection might be advanced that these cases which I have called "problems," were not such; that there was no reason why the animals should modify their behavior, and that what they did under the conditions of the experiment was nothing that they would not have done under normal conditions. This objection is met satisfactorily I believe by the following experiments.

A given arm was stimulated by encumbering it with a rubber tube, or by painting it with strong or dilute formalin or hydrochloric acid of different strengths. These trials, of which I made a great many, yielded very definite results. In only one case did an animal progress in the direction of the stimulated arm; in a few cases at an angle to it, using it as one of the propellers, whereas in the vast majority of cases it moved in the direction diametrically opposite the stimulated arm. If the stimulus was strong, the movements were very violent, but no difference in direction was noted in the case of weak and strong stimuli. Under ordinary circumstances it is impossible to predict the direction in which an ophiuran, all of whose arms are of the same size, will move, but if one of the arms be encumbered the prediction that the animal will move away from the stimulus will be verified in the vast majority of cases. I think it is justifiable to assert that the direction of progression has been determined in these cases, and if this is true there is a determining cause—a problem.

My second line of inquiry—whether encumbered animals showed a noticeable increase in the number of movements best adapted to solve the particular problem given, was begun by finding the percentage of crosses and contacts in the same animals under the two conditions stated. The results are summarized in Table V.

As contacts and crosses usually result from wavings I counted these in animal *II* unencumbered and with one arm encumbered. The results are summarized in Table VI

The general conclusion to be drawn from these experiments is that there is neither a decrease in the amount of time taken to solve the problem, nor an increase in the relative frequency of movements best fitted to solve it. In other words, the animals did not modify their behavior in accordance with the law of resolution, and consequently, so far as is objectively recognizable, learned nothing.

TABLE V

Animal	Arms Encumbered	Movements	Per cent Contacts	Per cent Crosses	Problems
I	0	231	4.0	2.0	0
I	1	202	3.9	1.9	3
II	0	267	13.8	10.8	0
II	5	553	9.2	11.2	5

TABLE VI

Animal	Arms Encumbered	Movements	Per cent Wavings
II	0	117	26.4
II	1	192	19.3

DISCUSSION

The facts which I have brought forward in the foregoing pages agree with those of Preyer and von Uexküll in showing that in problem solving the animal repeatedly changes its behavior, not persisting in a certain reaction when that is unsuccessful. If I venture to take issue with Preyer, and to assert that the behavior which both he and I observed does not warrant the conclusion that ophiurans are intelligent, I must rest my claim upon the validity of my interpretation of the facts, and this validity I shall now attempt to establish.

The behavior of Ophiura brevispina may be summarized by saying that this animal under normal conditions performs practically all the movements possible to a creature constructed as it is; that except for this limitation, its ordinary behavior is not predictable, and that even the righting movements, because of their variety occupy a place between the ordinary behavior and reflex behavior, for though more definite than the former, they are less precise than those highly perfected types of response which gave us our first idea of reflex action.

Regarding the manner in which ophiurans rid their arms of encumbrances, Preyer ('86, p. 125) says: "Aus den beschriebenen und ähnlich leicht zu variirenden Versuchen ergiebt sich zunächst, dass Ophiuren in 5-fach. verschiedener Weise sich gegen die beim Tasten und kriechen ihnen sehr hinderliche Bekleidung mit einem Schlauche vertheidigen: (1) streifen sie ihn ab durch Reibung am Boden wenn er locker ist, (2) schleudern sie ihn fort durch geissel. förmiges Hin und Herwerfen, (3) drücken sie ihn fest gegen den Boden mit dem freien Nachbararm, und ziehen den Arm aus dem dadurch fixirten Rohre heraus, (4) stemmen sie abwechselnd beide Nachbararme mit deren Zähnchen unten gegen dasselbe und schieben ihn ruckweise ab, (5) brechen sie durch Selbstamputation den Arm mit der unbequemen Bekleidung ab. Hilft dass eine Verfahren nicht, dann wird das andere angewendet. Sehe ich hier von dem letzten, der Autotomie, ab, von der noch die Rede sein wird, so beweist schon die 4-fache Art der Abwehr bei einem und demselben Individuum unter denselben aüsseren Verhältnissen, dass hier kein einfacher Reflex vorliegt. Vielmehr besitzen die Ophiuren die Fähigkeit sich ganz neuen, von ihnen noch niemals erlebten Situationen schnell anzupassen."

"Wenn Intelligenz auf dem Vermögen beruht, Erfahrungen zu machen, d. h. zu lernen, und das Erlernte in neuer Weise zweckmässig zu verwerthen, so müssen also die Ophiuren sehr intelligent sein."

Preyer's reasoning seems to be this: When encumbered on its arms the animal moves in different ways; failing to free its arms by these movements, it moves in other ways, and continues to change its movements until the encumbrances have been removed. The animal thus exhibits the process of discovery by elimination, learning in other words, and is therefore intelligent.

If this indeed be learning, then all movements which any organism may under any circumstances execute are outward signs

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THE PHYSIOLOGY OF NEMATOCYSTS

By
O. C. GLASER AND C. M. SPARROW

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THE PHYSIOLOGY OF NEMATOCYSTS[1]

BY

O. C. GLASER AND C. M. SPARROW

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INTRODUCTION

Grosvenor ('03) has given a brief review of the different theories which have been invented to explain the discharge of nematocysts, and has himself proposed a view which in the present state of our knowledge seems the only one worth careful consideration. Grosvenor's theory is that the discharge of nematocysts in Cœlenterates, and in those animals which derive their nematocysts from them, is brought about by osmotic pressure. His evidence is as follows: Cerata of Eolis immersed in Calberla's fluid, extrude large numbers of undischarged nematocysts. If the fluid is diluted with sea-water, the threads of the capsules are everted. Similar results were obtained when cerata were plunged into "fairly

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[1] Contribution from the Zoölogical Laboratory of the University of Michigan, No. 122.

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strong" solutions of sugar or salt. Here too the nematocysts v not discharged, but when the preparations were subseque washed out with distilled water, the nematocysts exploded. G venor also experimented with the tentacles of actinians. W these were plucked off, and quickly thrust into a 50 per cent s tion of sugar, and teased into small fragments, though many charged nematocysts were found, pieces in which none had charged were common enough. Such fragments were isol and kept for from 24 to 72 hours. The nematocysts remai undischarged until the sugar solution was washed out with wa when approximately 20 per cent of the capsules discharged th selves.

"These facts," says Grosvenor, "seem to show that the charge of nematocysts is due to osmosis. The capsule appare contains a solution of such strength that it takes up water f such a weak solution as sea water, but not from the protoplasn the nematocytes, or the fluids in the alimentary canal of Æol or from any of the other solutions mentioned above."

Our interest in the history of the nematocysts of Æolids, ('02; led us in the summer of 1908, to undertake a careful investigat of this subject. The work was begun by the Senior author the Zoölogical Laboratory of the University of Michigan, was brought to a practical completion in the Marine Biologi Laboratory at Woods Hole. To the Director, Prof. F. R. Lil we are indebted for the use of a room in the laboratory.

MATERIAL AND METHODS

The material used for the experiments and for the developm of methods consisted of Hydra, Metridium, Physalia, and M tagua. Experiments were made with the nematocyst bear tissues of these animals, and also with the isolated stinging c sules. The methods used for isolating them were peptic a auto-digestion at 35° C., and maceration in sea-water to wh crystals of chloretone had been added. In the case of the pe digestions, all the tissues except the nematocysts, were dissol in a solution composed of 4 cc. HCl; 1000 cc. H_2O Dist.; a

10 gr. flaked pepsin. The digestive processes took place quite rapidly, in some instances being complete within 24 hours. The solutions were then centrifugated, and there was obtained a thick sediment, composed, in the case of Physalia especially, of countless isolated, undischarged nematocysts. Many discharged ones also were found, but these formed a minority. The same methods were employed in the case of the auto-digestion.

Experiments to be described later, showed that although these methods are adequate, the nematocysts are changed in certain ways by these processes, and we therefore resorted to the maceration method referred to. For this purpose the acontial filaments of Metridium were found extremely good as they are composed of immense numbers of nematocysts held together by a minimum of other tissues. In sea-water to which crystals of chloretone are added slowly from time to time for a period of about 12 hours, the acontia break down, forming a somewhat glutinous mixture in which free undischarged nematocysts occur in great abundance. These were in excellent condition for some of the purposes for which we used them.

In order to store nematocysts for later use, and this was necessary as we secured only one specimen of Physalia, and that early in the summer, we dessicated some of the sediment secured from the centrifuge, preserved some in glycerine, some in sea-water, and some in salt solutions of various concentrations. Under these circumstances the material keeps perfectly well, and can later be used for experimental work.

During the course of the experiments carried out at Wood's Hole it became desirable on several occasions to separate the discharged from the undischarged nematocysts, and also to isolate individual capsules. The former was accomplished in two ways, sometimes by means of what might be called a "capillary filter," the discharged nematocysts failing to be drawn up into a capillary tube just large enough to admit undischarged ones; sometimes by taking advantage of the somewhat lighter specific gravity of the exploded capsules. In a dish containing both discharged and undischarged nematocysts, the former come to lie above the latter and may be completely removed by means of a small glass hook.

Isolation of individual capsules, whenever desirable, was accomplished by means of a capillary tube filled with a suspension of nematocysts. By spreading, from the mouth of such a tube, small drops on a glass slide the nematocysts may be distributed so that each drop contains only a few, or perhaps only one. The drops can then be numbered, and the history of the individual nematocysts followed for any desired length of time. A moist chamber was frequently used to prevent the drops from drying.

EXPERIMENTS WITH MONTAGUA

The nematocysts of Montagua are derived from its prey, Tubularia crocea. The details in this transfer, are being reserved for another paper. For the present purpose it is necessary to know only that these derived nematocysts are stored by certain entodermal cells, the cnidophages, inside the cnidophores of the dorsal cerata, and that when each storage cell has engulfed a certain number it loses its cellular characters and becomes converted, possibly with the assistance of certain neighboring interstitial cells, into a thin transparent bag, the cnidocyst. These loaded cnidocysts lose their connection with the basement membrane to which in earlier stages they are attached, and come to lie free in the lumen of the cnidophore in the distal end of the appendage.

Under certain circumstances the elimination of cnidocysts filled with stinging capsules may be observed under the microscope. If the animal is stimulated mechanically, chemically, or best of all thermally, the extrusion of the cnidocysts takes place. They are shot out of the cnidopores at the tips of the dorsal cerata, not by violent contractions on the part of these appendages, but by unobservable contractions probably of the musculature of the cnidophore. Relaxation of this musculature immediately around the cnidopore is either incomplete, or if complete, is not great enough to allow the easy passage of the cnidocysts. These, while elimination is going on, are often much distorted, but as soon as the pressure from the walls of the cnidopore is relieved, they become spherical. At times they make their appearance as clear bubbles blown by the cnidopore, and they may remain in this condition until they have been almost completely extruded, when the nematocysts begin to shoot into the visible portion, either one by one, or in groups. When this has happened, the cnidocysts leave their positions at the mouth of the cnidopore, usually on account of the movements of the animal or of its appendages, and may float freely in the water nearby, or may remain adhering to other regions of the ceras.

The nematocysts so extruded, in many instances discharge inside of their enclosures and as their threads penetrate through the wall of the cnidocyst, this may come to resemble a "sperm-bundle," with filaments radiating in all directions. Ultimately the cysts burst, and set free their discharged contents. This, however, is not the usual history—ordinarily the bursting of the cnidocyst and the explosion of its nematocysts take place at the same instant. The questions therefore arise: Why do the cnidocysts burst, and why do the nematocysts discharge? There are involved no living tissues which might be responsible; the nematocysts are not living things, and their enclosing cnidocysts are also dead.

A simple experiment gives the answers. If Montagua is stimulated thermally in a concentrated sugar solution, the elimination of cnidocysts takes place as described, only as soon as they come into contact with the surrounding medium they shrivel. None of the cysts burst, and none of the nematocysts discharge. If now the sugar solution is replaced by distilled water, the cnidocysts swell and burst, and the nematocysts discharge. Discharge, however, is rarely complete; a few nematocysts in every collection of them fail to discharge under circumstances under which the majority explode.

If this experiment is modified, and the elimination is forced to occur in distilled water, the bursting of the cnidocysts and the discharge of the nematocysts take place so quickly, that it is impossible to be more than aware of the processes. Even under these conditions some nematocysts may remain undischarged.

The most plausible explanation of these results is that the bursting of the cnidocysts, and the discharge of the nematocysts are due to absorption of water; that introduction into a medium of higher osmotic pressure than the contents of either the cnidocyst, or the nematocysts, results in the abstraction of water, and that for this reason, the former shrivel and the latter remain intact. Why some of the nematocysts fail to discharge when the majority explode, will be discussed in connection with later experiments.

EXPERIMENTS WITH THE TENTACLES AND ACONTIA OF METRIDIUM

The results of Grosvenor, and those just described, lend strong support to the idea that the discharge of nematocysts is due to osmosis, and while none of our experiments seem to indicate that this idea is erroneous, the study of the living tentacles and acontia of Metridium, shows that the matter is not quite as simple as might be supposed. In nematocyst-bearing tissues, another factor must be reckoned with, the living nematocyte, the cell which makes the nematocyst and encloses it.

The living tentacles, as well as the acontia of Metridium may be removed without discharging the nematocysts; this can be done very easily in the case of the tentacles, not quite so easily with the acontia, but even in this instance, an abundance of intact threads or pieces of threads, is readily obtained. These can then be treated in various ways, and the behavior of the nematocysts studied.

In certain media, many of the nematocysts leave their natural positions in the mother tissue, but do not discharge; media of much higher osmotic pressure than sea water, may bring about discharge, and heat, electricity and mechanical pressure are effective. At first sight these results seem to be strongly antagonistic to the osmotic theory, but careful analysis of them, either changes all of these data into positive supports or at least disarms them.

In the following table is given a résumé of the details of the experiments on the effects of various media and stimuli on living nematocyst-bearing tissues. The material used is mentioned in the first column; the treatment given it, in the second; whereas the effects on the nematocysts are recorded in the third and fourth columns. The word extrusions is used to designate those instances in which nematocysts, without exploding, left their normal positions in the mother tissue. Such extrusions are due either to a breaking down of the surface of the tentacles or acontia when exposed to certain media, or in some cases to contraction. All of the experiments were repeated several times, and some, many times, so that the reports are based on the behavior of thousands of nematocysts.

Material used	Treatment	Extrusions	Explosions
Metridium tentacles...........	sea-water	none	none
acontia	sea-water	none	none
tentacles..................	distilled water	many	many
acontia....................	distilled water	many	many
tentacles..................	saturated sugar solution	none	none
acontia....................	saturated sugar solution	none	none
tentacles..................	idem followed by H_2O dist.	many	few
acontia....................	idem followed by H_2O dist.	all	none
tentacles..................	saturated sodium chlorid	many	many
acontia....................	saturated sodium chlorid	few	many
tentacles..................	Kleinenberg's picro-sulfuric	few	many
acontia....................	Kleinenberg's picro-sulfuric	none	all
tentacles..................	sublimate-acetic	few	many
acontia....................	sublimate-acetic	none	all
tentacles..................	saturated mercury bichlorid	none	none
acontia....................	saturated mercury bichlorid	none	none
tentacles..................	acetic acid	many	many
acontia....................	acetic acid	none	all
tentacles..................	hydrochloric acid	few	many
acontia....................	hydrochloric acid	none	many
tentacles..................	ammonium hydroxid	few	many
acontia....................	ammonium hydroxid	none	all
tentacles..................	95 per cent alcohol	none	many
acontia....................	95 per cent alcohol	many	many
tentacles..................	chloroform	none	many
acontia....................	chloroform	none	many
tentacles..................	ether	none	many
acontia....................	ether	many	many
tentacles..................	chloretone	none	many
acontia....................	chloretone	many	many
tentacles..................	mechanical pressure	many	many
acontia....................	mechanical pressure	many	many
tentacles..................	heat 100° C.	many	many
acontia....................	heat 100° C.	many	many
tentacles..................	heat 0 °C.	none	none
acontia....................	heat 0° C.	none	none
tentacles..................	alternating current	many	many
acontia....................	alternating current	none	all

It is not necessary to give a detailed analysis of the experiments summarized in Table I. In general they indicate that specific chemical effects are not involved, and further that any theory which attempts to explain the discharge of nematocysts, must take account of the nematocyte. This particular phase of the subject, however, can be more profitably discussed after the experiments on isolated nematocysts have been reported. These also will explain some of the above results which at first sight may appear puzzling.

EXPERIMENTS WITH ISOLATED NEMATOCYSTS

A nematocyst is a membranous capsule, one portion of which is prolonged into a thread, ending in a point. In its undischarged state, this thread is introverted, and is stored inside the capsule of which it is an organic part. In addition to the visible filament, the capsule contains certain invisible chemical substances.

On the basis of this knowledge, we may make certain assumptions regarding the causes that bring about eversion of the thread, and these assumed causes can then be tested experimentally. We may assume that in order to bring about discharge, it is necessary to raise the internal pressure of the capsule to a point at which it can overcome the effect due to the uniform external pressure to which the capsule is subject, plus whatever resistance to eversion is offered by the construction of the capsule itself. We may assume further, that the capsule is a membrane, semi-permeable to aqueous solutions, and that it contains substances capable of absorbing water. We may assume also that the membrane is specifically permeable to certain ions, although, if the results of the experiments can be explained without this assumption, postulation of specific permeability becomes unnecessary.

These assumptions were tested experimentally. The results which have been actually obtained appear to be explicable by any one, or any probable combination, of the following factors: increase of internal pressure; decrease of external pressure; reduction in the resistance to eversion due to the construction of the capsule.

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Mechanical Pressure

The effect of mechanical distorting pressure was studied by mixing the nematocysts of Physalia or the tentacles of Metridium with granulated salt and grinding the material between glass plates. Sometimes the salt was omitted, and ground glass plates were used. After treatment in this manner, the nematocysts were examined. In those cases in which salt was used, this was dissolved before observations on the results of the treatment could be attempted. In this way a considerable mechanical distorting pressure was applied to the individual capsules, and though the nematocysts of Metridium, on account of their minute size and their delicacy, gave inconclusive results, those of Physalia gave very positive ones. Many partial discharges were obtained. Pressure on the cover glass of a preparation of Physalia nettles also causes many partial discharges. Such pressure as was used in these experiments distorts the capsules, and is effective because the internal pressure of the nematocysts is raised by distortion.

Uniform External Pressure

That the effects of distorting pressure have been correctly interpreted, is clearly shown by the effect of high uniform external pressure. Such pressure was applied by allowing the nematocysts to be drawn up into a capillary tube provided at one end with a reservoir filled with mercury. The open end of the tube was then sealed and the mercury made to expand.

The pressure obtained in this manner, calculated from the contraction of the air bubble inside the tube, and from the bursting strength of the tube, was from 50 to 100 atmospheres. No nematocysts ever discharged when treated in this way.

Solutions

In Table II are presented in condensed form the results of experiments undertaken to discover the effects on isolated nematocysts of the same solutions which had previously been employed on the living tentacles and acontia of Metridium. The isolated nematocysts of Physalia were not used in this series of experiments for reasons which will become clear later—all the results presented in this section are based on isolated Metridium nematocysts secured by the maceration method.

TABLE II

Solution	Effect
Sea water	none
Distilled water	complete instantaneous discharge
Saturated sugar solution	none
Idem followed by H_2O dist	partial and slow discharge
Saturated sodium chlorid	none
" strontium chlorid	none
" zinc sulfate	none
" magnesium sulfate	none
" sodium carbonate	none
" potassium carbonate	none
Kleinenberg's picro-sulfuric	complete instantaneous discharge
Sublimate acetic	complete instantaneous discharge
Saturated mercury bichlorid	none
Acetic acid	complete instantaneous discharge
Hydrochloric acid	complete instantaneous discharge
Ammonium hydroxid	complete instantaneous discharge
95 per cent alcohol	none
Chloroform	none
Ether	doubtful
Chloretone	none

In every case, except that of sea-water, dilution occurred when the reagents listed above were brought into the fluid of the suspensions. The error due to this, however, is of no consequence in the present connection. With one or two exceptions, to be discussed later, the same solutions, effective in bringing about the discharge of nematocysts within their mother cells, are capable of causing the same effects when the nematocysts are isolated.

An examination of the table shows that these results give strong support to the osmotic theory. The positive effects of distilled water, of dilute acids, such as Kleinenberg's picro-sulfuric, and sublimate acetic, and the negative results from the use of the saturated solutions of sugar, and of sodium, strontium, magnesium, and potassium and mercury salts, are all to be expected. Some of the other results, however, require a word of comment.

Alcohol, ether, even if effective, chloroform and chloretone, used because employed in the previous experiments on living tentacles and acontia, bear neither way on the osmotic theory. The action of strong acids and of ammonia remain to be explained. Acids are chemically very active, and it is conceivable that upon penetration into the nematocyst they affect a decomposition of the intracapsular contents, thus increasing the number of molecules present, and hence the internal pressure. Since the H ion is the active one, it is possible that the membrane is specifically permeable to it.

The effect of acids, however, may be explained in the same way as the action of ammonia. The latter is effective possibly on account of its power of disintegrating tissues. If the capsule is weakened at the point where the thread is introverted—a point normally weak—eversion is likely to occur, for as will be shown later, the capsular contents themselves exert a high pressure.

The effect of distilled water on nematocysts which have been treated with a saturated solution of sugar, is due to the fact that sugar probably "gums up" the pores of the capsules. Other agents do the same thing, and it is for this reason that the Physalia material was not used, although in the course of time it would probably have given the same results. This is indicated by the following observations.

In suspensions made in distilled water, from dessicated Physalia nematocysts, as well as from those preserved in glycerine, it was noticed that the older the suspension, the greater the number of completely discharged nematocysts. This increase was so great that in the course of several days the exploded ones began to outnumber those intact. This phenomenon pointed to slow osmotic interchange between the capsular contents and the surrounding medium. Grosvenor, in dealing with pieces of actinian tentacle teased up in a half concentrated sugar solution, found, when the sugar is washed out with distilled water, that "never more than approximately 20 per cent" of the nematocysts discharge themselves. Had he waited, he would no doubt have found the percentages much higher. The results obtained from the macerated material, in which discharge was complete, and also instantaneous, show that media, such as glycerine, and sugar solutions, either clog the pores, and make diffusion a slow process, or else make the eversion of the thread so difficult that a higher pressure than the normal one is needed to bring about explosion. Both of these causes might be operative together, and, in addition, it must be remembered that the digestive processes in themselves might have the effects suggested, and might also alter the constitution of the intracapsular contents.

Hypertonic Solutions

If the results already described support the osmotic theory, the effect of hypertonic solutions completely demonstrates its correctness. Not only do nematocysts fail to explode in such solutions (Table II) but if left in them for a number of days, they can be made to discharge in media too concentrated to bring about the explosion of normal nematocysts. These results, which will be referred to again in another connection, can be explained only on the assumption that by slow transfusion the intracapsular contents are so changed by the hypertonic solutions, that the nematocysts become able to absorb water from media more concentrated than those toward which they are normally osmotically neutral.

Negative External Pressure

If the explosion of nematocysts is due to pressure from within outward, as the osmotic theory requires, and as the effects of distorting pressure seem to indicate is true, it follows that a negative external pressure might result in explosion, particularly if, as is conceivable, the capsules are in a state of tension. Negative pressure of one atmosphere, produced by suction, gave entirely negative results. This failure, however, is not traceable to a mistake in principle, but to the insufficiency of the negative pressure. The osmotic pressure of sea-water is in the neighborhood of 22 atmospheres (Garrey '04), and as will be shown later, the pressure of the intracapsular fluid must be about the same. It was found that at ordinary temperatures, practically all of the nematocysts discharged in a solution of 70 per cent distilled and 30 per cent sea-water, whereas practically no explosion occurred in a mixture of 60 per cent sea-water and 40 per cent distilled. Since this latter dilution gives $\frac{40}{100} \times 22$, or 9 atmospheres as the minimum pressure required to bring about explosion, it is easy to see why a simple vacuum proved wholly inadequate.

Heat

Low temperatures hinder discharge, and make it necessary to employ solutions of much greater dilution than are needed at ordinary temperatures. High temperatures on the other hand greatly facilitate discharge, and make it possible to explode nematocysts in media more concentrated than sea-water. These effects in all probability are due to a combination of factors.

In the case of low temperatures, the capsule probably contracts, and thus renders more difficult, not only the absorption of water, but the actual extrusion of the thread through the narrow opening out of which it must be everted. The increase in the viscosity of the medium due to the lowering of the temperature is also a considerable quantity. When dealing with high temperatures on the other hand, the viscosity of the surrounding medium is reduced; the expansion of the capsule not only makes absorption easier, but also the actual process of eversion; further the pressure within the capsules must be raised, partly on account of the increased speed of the molecules in the intracapsular fluid, partly on account of an actual increase in the number of molecules present, for Portier and Richet ('02) have shown that the hypnotoxin breaks down at 55° C.

Alternating Current

Although capable of causing the discharge of nematocysts imbedded in their living mother tissues, when applied to the isolated capsules the alternating current proved ineffective. The result is explained by the fact that an alternating current is incapable of changing the concentration of the solution through which it passes, on account of the compensatory effect of the rapid reversals in direction.

RATE OF EXPLOSION

The fact that the rate at which explosion takes place may be greatly modified by treating the capsules with glycerine and sugar, suggested the possibility of controlling the eversion of the thread in other ways. If the osmotic conception is correct, a moderate increase in the concentration of a solution should reduce the speed of the discharge, and a great increase should prevent explosion altogether. Both of these effects were obtained, though under influence of heat, the capsules continued to discharge in media too concentrated to allow explosion at ordinary temperature.

The reduction in the speed obtained by the use of concentrated sea-water, and other media of high osmotic pressure, made possible certain observations on the eversion of the thread which are in complete harmony with the osmotic theory. In such media, when the dilution is just sufficient to bring about explosion, one can see that during the process of eversion, the thread is cast out suddenly, but only to about two-thirds its length. A brief period —less than a second often—of inactivity, due no doubt to the immediate relief of pressure, ensues, and then the remainder of the thread is everted. To observe this effect one must use a medium only a trifle less concentrated than that from which the nematocysts were taken.

VARIATIONS IN THE EXPLOSIVE PRESSURE

In practically all of the experiments on isolated nematocysts, it was noticed that not all of the threads are everted under circumstances under which most of the capsules explode. At certain concentrations no explosions occur; if the solution is diluted, a few incomplete or slow discharges occur; further dilution increases both the number and the rate of the discharges, and finally a point is reached at which the great majority explode. Even here, however, a few remain unaffected unless the medium is diluted still further. It follows from this that the pressure necessary to explode nematocysts instantaneously varies with the individual capsule, and as these differences occur in nematocysts prepared by the digestion as well as the maceration methods, it is safe to conclude that the observed facts are normal.

It is conceivable that the porosity of the capsular wall may vary with its age, or may vary independently of this, and the same thing is true of the intracapsular contents. Either of these possibilities would account for the facts. It is also true that other slight differences in the construction of the capsules might affect the pressure needed to explode them, and possibly also, not all of them are in equally good working order. In the eversion of a barbed thread, like that characteristic of the nematocysts of Metridium, it would seem that there is ample opportunity for entanglements, capable of being loosened or broken only by increased pressure from within.

APPLICATIONS OF THE OSMOTIC THEORY TO ÆOLIDS AND CŒLENTERATES

Æolids

The experiments described can leave no doubt that osmotic pressure can account for all of the observed facts. The question now arises, why do the nematocysts of nudibranchs discharge on coming into contact with sea-water, whereas those of cœlenterates remain intact?

While enclosed within their mother cells in the cœlenterate, the nematocysts must be osmotically neutral toward their cellular environment; and since they themselves are neutral toward sea-water, it follows that we must consider the nematocyte also osmotically neutral toward its external environment. This neutrality must be disturbed by the sojourn of the nematocysts within the bodies of the nudibranchs, or some other factor must enter to counteract it. After careful consideration of the possibilities that suggest themselves, we have discarded all but one: by slow transfusion, the contents of a nematocyst osmotically neutral toward sea-water, may be changed, so that it becomes capable of absorbing water, and consequently of raising its internal pressure to the exploding point. To test this idea, isolated nematocysts of Metridium were treated with a saturated salt solution for four days. After this time sea-water, which is osmotically neutral toward freshly isolated nematocysts, was as effective in bringing about discharge as distilled water is when applied to unmodified capsules.

Cœlenterates

A comparison of the results obtained from isolated nematocysts, and from those imbedded in their living mother tissues, suggests that the explanations which hold good for the former class hold equally good for the latter; that in the one case we are dealing with an osmotic interchange directly between the capsule and its surrounding medium; in the other case between the nematocyte and the medium, and that the permeability of the cell to the various reagents used, is such that for practical purposes the nematocyte is non-existent. It must be apparent that in most cases it is impossible to show that this, as a generalization, is incorrect, nevertheless, we believe that it is incorrect, and that the nematocyte, the mother cell of the nematocyst, has something to do with its discharge, possibly not under all circumstances (see Tables I and II) but certainly under some, and perhaps always when the nematocyst is discharged in response to stimuli normal in the lives of cœlenterates.

The efficacy of the nematocyte as a factor in the normal discharge of a nematocyst can be shown in at least three ways. A saturated solution of sodium chlorid is incapable of bringing about the explosion of isolated nematocysts. This, however, is not true when the same solution is applied to the living tentacles and acontia of Metridium. (Table I.) Under this treatment a complete discharge of all the nematocysts occurs. The alternating current, when applied to isolated nematocysts, is ineffective, but when applied to fresh tentacles and acontia, it brings about the discharge of all the nematocysts present.

These two experiments suggest that the cell is effective, and that the reason why the nematocysts explode under the conditions named, is because the nematocyte is stimulated to do something which brings about discharge. The correctness of this inference can be established, if without destruction, the nematocyte can be eliminated from possible participation in the chain of events. This can be done by narcotization. The most effective agent to use, if used with moderation and care, is chloretone. If the tentacles and acontia of Metridium are narcotized with chloretone, saturated sodium chlorid, 95 per cent alcohol, and chloroform, all of which act as stimuli under normal conditions, do so no longer, and the nematocysts enclosed by their anesthetized mother cells fail to explode. These results seem to point conclusively toward the nematocyte as a factor in the normal discharge of a nematocyst, and this in spite of the fact that distilled water, Kleinenberg's picro-sulfuric acid, sublimate acetic, acetic acid, ammonium hydrate, and ether, are as effective on narcotized material as on normal. All of these liquids are highly penetrating, or contain very penetrating elements, or have specific gravities, so little above that of distilled water, that they act under all circumstances, as though the mother tissues, normal or narcotized, were not there. Heat also is effective when applied to narcotized nematocytes, either because the nematocysts under its influence absorb water, or because their contents break down (p. 373.). In addition to the explanations suggested, it is possible that a nematocyte narcotized sufficiently to be unresponsive to certain stimuli, it is not necessarily sufficiently under anesthesia to render all stimuli ineffective.

Why then do the nematocysts of Cœlenterates explode under normal conditions? Since they are completely enclosed by the fluid contents of the nematocytes, contraction on the part of these cannot be effective, since a uniform external pressure, no matter how high it may be, is incapable of causing discharge. The possibility of a distorting pressure produced by the nematocyte is not absolutely ruled out, though we know of no mechanism by which it might be produced. Appeal to undiscovered cytoplasmic fibrillæ might be made, but with little profit. The osmotic theory on the other hand can be applied here also even if direct evidence is still wanting.

In making an application of the osmotic theory two possible factors suggest themselves, and these, operative singly or together, will account for the facts. It is conceivable that when stimulated, the nematocyte suddenly generates heat; it is also conceivable that the cytoplasm around the nematocyst undergoes chemical and physical changes of such a nature that the capsules are enabled to absorb water, and to raise their internal pressures to the exploding point. Particularly if heat is liberated at the same time that "dilutation" occurs, this theory offers no insurmountable difficulties. The time element need not be considered, for the chemical and physical changes which stimulation sets up in a muscle occur very quickly, and when the proper reduction in the concentration of the surrounding medium has been made, a nematocyst explodes instantaneously.

THE PHYSIOLOGICAL EFFECTS OF NEMATOCYSTS ON OTHER ORGANISMS

On the chemical side, it has been shown by Portier and Richet ('02) that an aqueous extract made from twenty-nine of the tentacles of a Physalia contained enough poison to kill a pigeon within an hour after injection. Curiously enough no inflammation was set up; irritability and temperature were reduced and diarrhœa frequently set in. These experiments, together with the observation that frogs or fish, when placed in contact with the filaments of Physalia, make no attempts to escape, led Portier and Richet to name the poison involved, hypnotoxin. Very little is known regarding its chemical nature. It is destroyed by a temperature of 55° C.; can be precipitated with alcohol; and is non-dialysable. Von Fürth ('03) who gives a résumé of Portier and Richet's work, adds that it is necessary to assume that the nettles, in addition to the hypnotoxin, contain a violent "Reizgift," which accounts for the inflammations, which in spite of the observations quoted above, have been observed in other cases.

On the physical side, the conclusions of Iwanzoff ('96) and the earlier ones of Möbius ('66), are opposed in certain important respects. Iwanzoff states that the physiological effects of the nettles are due to the numerous poisoned threads which surround and penetrate the victim. Möbius distinctly opposes this view as well as others which are current. For instance he considered the idea that the hairs on the filaments are "back hooks," a mistaken one, not only because in the ripe thread they stand out at right angles, but also because they are too delicate to serve the function attributed to them. Möbius might have added that many types of functional nematocysts are devoid of these barbules.

As for the ability of the thread to penetrate into the tissues of the victim, Möbius considered this impossible, in the first place because the thread "unrolled" too slowly, and in the second place, because its point does not strike the victim. In fact, the point is the last portion of the thread to be everted, and it is, of course, cast out with less force than any other part. The great delicacy of the point was also offered as evidence against the validity of the current belief.

The nettling sensations produced by nematocysts, Möbius did not attribute to the minute punctures made by the filaments, but to the fact that these are saturated with some chemical, which on coming into contact with the skin, produces irritation. This chemical remained undetermined, but relying entirely on microchemical tests, Möbius concluded that it is neither formic acid, nor any other acid.

The existence of hypnotoxin seems to be fairly well established, whereas there is considerable doubt about the inferred "Reizgift." All the phenomena which the "Reizgift" could explain, seem to me to be explicable on the assumption that the filaments, contrary to Möbius' conception of their powers, do actually penetrate the tissues. The idea does not seem to have occurred to him that a thread might penetrate a tissue before being completely everted; he does not seem to have realized at what immense pressures the discharges occur; nor, if he knew of such instances, did he recall that, given sufficient velocity, a stem of hay will shoot through a pane of glass.

Observations, as well as experiments, bearing on the penetrating power of the filaments were made. Thus the acontia of Metridium were placed on fresh tissue taken from a clam, and the nematocysts were then discharged by fixing the preparation in sublimate acetic. The tissues which had been "shot" in this way were then sectioned, and in several regions it was possible to trace the filaments of the nematocysts through the epidermis into the muscle and connective tissues below. Most of the threads however, did not enter the tissues, but seemed to have been warded off, and in the sections lie tangential to the epidermis. This is what might be expected, for unless a filament penetrates while it is at the height of its speed, it fails to make a puncture at all, for the extreme end is everted too feebly, just as Möbius says.

Direct observations on the behavoir of the nematocysts of Montagua, while still enclosed within the cnidocysts, are much more favorable for the elucidation of this question. We have reported in an earlier section that the nematocysts may explode before the cnidocyst bursts, and that the discharged filaments are capable of penetrating through their enclosing membrane.[2] The length of the discharged filament; the position of the nematocysts inside the cnidocyst, and the diameter of the cnidocyst, make it absolutely impossible for the filaments to penetrate the membrane at any other than the early stages of eversion. The cnidocysts are not large enough to allow anything else; nevertheless the filaments penetrate them, which is exactly what they should do if the osmotic theory of discharge, and the considerations brought forward in the preceding paragraphs, are correct.

SUMMARY

1 The material used consisted chiefly of the living tentacles and acontia of Metridium, and nematocysts, isolated, by digestive and other methods, from Metridium and Physalia.

2 The discharge of nematocysts is due to internal pressure. This pressure may be raised to the exploding point by osmosis and by distortion.

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[2] Two months after this paper was written Toppe (Zoologischer Anzeiger Bd. xxxiii, Nos. 24/25) published an account of his very careful observations on the manner in which nematocysts discharge, and showed conclusively that the nettling threads are able to puncture the chitinous covering of a Corethra larva.

3 The explosive pressure varies with the individual nematocysts, and with circumstances. It may be artificially altered. This fact explains why the nematocysts of Æolids explode in sea-water, whereas those of Cœlenterates do not unless the nematocyte is stimulated.

4 It is impossible to show that the nematocyte is a factor in the discharge of the nematocysts of Cœlenterates under all circumstances. Nevertheless, this is true under some circumstances, and perhaps always under the conditions which are normal in the lives of cnidaria.

5 The osmotic theory, originally advanced by Grosvenor on very limited evidence, is absolutely supported, as far as isolated nematocysts are concerned, and may be applied to the normal discharge of stinging capsules in Cœlenterates, if we suppose that stimulation of the nematocyte inaugurates changes which result in the liberation of heat or in lowering the concentration of the intra-cellular medium immediately surrounding the nematocyst. Both heat and dilution may be operative.

6 The filaments of nematocysts are capable of penetrating the tissues of other animals, contrary to the opinion of Möbius, but in order to do this, must make their punctures before eversion is complete.

Zoölogical Laboratory
University of Michigan
November 25, 1908

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PAMPHLET COLLECTION

The Formation of Habits at High Speed

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From the Zoölogical Laboratory, University of Michigan

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THE FORMATION OF HABITS AT HIGH SPEED[1]

OTTO C. GLASER

From the Zoölogical Laboratory of the University of Michigan

WITH TWO FIGURES

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INTRODUCTION

The method by which habits are ordinarily educed consists essentially in presenting a problem whose solution depends on the slow, and often painful suppression of irrelevant actions, and the survival of only those that count. The results so achieved are invaluable, but from the nature of the case difficult to verify. So much time is required that students of all classes are apt to be told in words, rather than by actual experiments, what has been accomplished in this interesting field. Habits formed slowly and gradually are not only incapable of quick demonstration, but the "slow method" leaves altogether untouched, a wide range of behavior. Animals do not always act slowly; they do not always overcome, with deliberation and care, the difficulties that block the road to food and comfort; indeed, in nature there are many problems whose solution must be accomplished at once with the utmost rapidity, and to the swift alone is the race.

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[1] Directly, as well as indirectly, I am indebted to Miss Frances J. Dunbar, and to Miss Nina Gage, for many of the results on which the present communication is based.

METHODS

The animals used were white rats of different ages, and the general method, a modification of the time-honored labyrinth. Instead of the usual form, however, I constructed a zinc tank, 2 feet, 1 inch square, and 6 inches deep, and covered it with coarse-mesh zinc netting, in the exact center of which a circular opening, fitted with a cylindrical shoot, serves as an entrance for the animals. At each of the four corners the cover, which is firmly clamped to the sides of the tank, has a small hinged door that can be opened at will, or made fast, likewise by means of clamps.

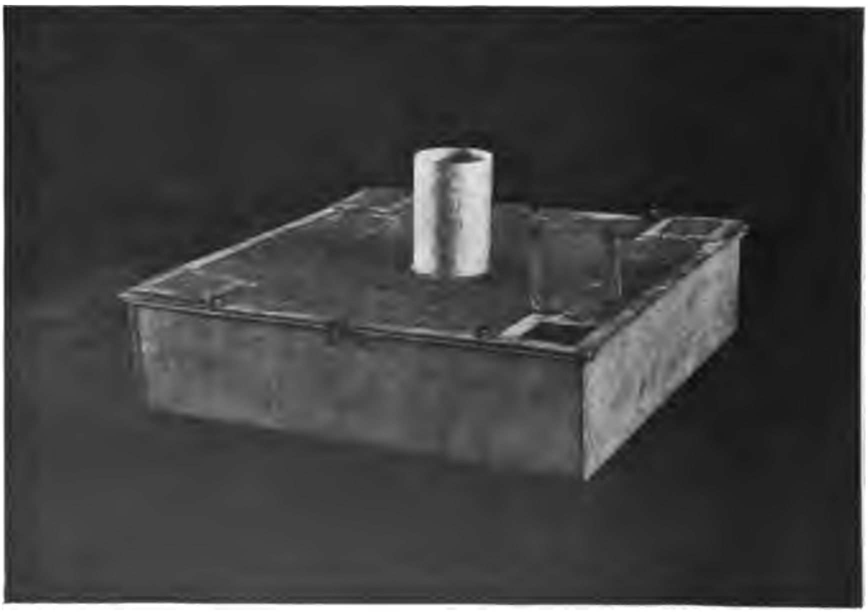

FIG. 1. Tank. Photograph by Miss Frances Dunbar. Dimensions 2 feet 1 inch square; depth, 6 inches; diameter of funnel, 4 inches; height of funnel, 6 inches; exits, 3 inches square.

During the experiments, the tank was filled, sometimes with warm, sometimes cold water; salt was added on some occasions, whereas on others additional stimuli were administered while the animals were falling through the shoot or after they had emerged through the open door. As there are four openings, any one of which may be made the correct one, and as the contents of the tank may be varied in many ways, and the experiences on entering and emerging complicated as much as one desires, the tank is in a real sense, labyrinthine, although simple in construction.

GENERAL RESULTS

It is needless to say that an inexperienced animal suddenly thrust into a tank of water makes strenuous efforts to escape. Under the conditions of the experiments it is not surprising that a high degree of variability should attach to the several attempts of an animal forced to undergo the experience of the tank half a dozen times in succession. Nevertheless there is an underlying regularity, for the time taken to escape in 83 per cent of the cases is less at the last attempt than at the first, whereas in 16 per cent it is greater, and in only 1 per cent unchanged.

Time records, while the most convenient form of registration, are nevertheless not the only ways in which the formation of a habit manifests itself. Very much to the point in this connection are tracings of the actual pathway pursued in escaping. Six such graphic representations are given in fig. 2, and show conclusively that the first turn of the path that led to the first escape occurs, often much abbreviated, and regardless of advantage, in nearly all of the succeeding trials.

In experiments into which so many complicating factors enter a regular and machine-like progress toward perfection cannot be expected, and its failure to appear is clearly shown in series A and B. Records C and D, however, illustrate distinctly how the path followed was simplified until in the last trials it became to all intents and purposes a straight line. Hand in hand with this was a reduction in the amount of time taken to effect the escape.

Two facts of considerable interest are to be read in these tracings. In series B, C, D, and E, the pathways are without exception dextral; in series E, on the other hand, all the turns made are sinistral. A directive factor seems to be operative, but analysis shows that it may not be simple. A considerable number of tests was made with blindfolded animals, and with young whose eyes were not yet open. The results in general indicate that certain individuals have a natural disposition to turn to the right, rather than the left, in swimming, whereas in others the reverse tendency is equally marked. I have made no experiments to determine whether this tendency is due to differences in the semi-circular

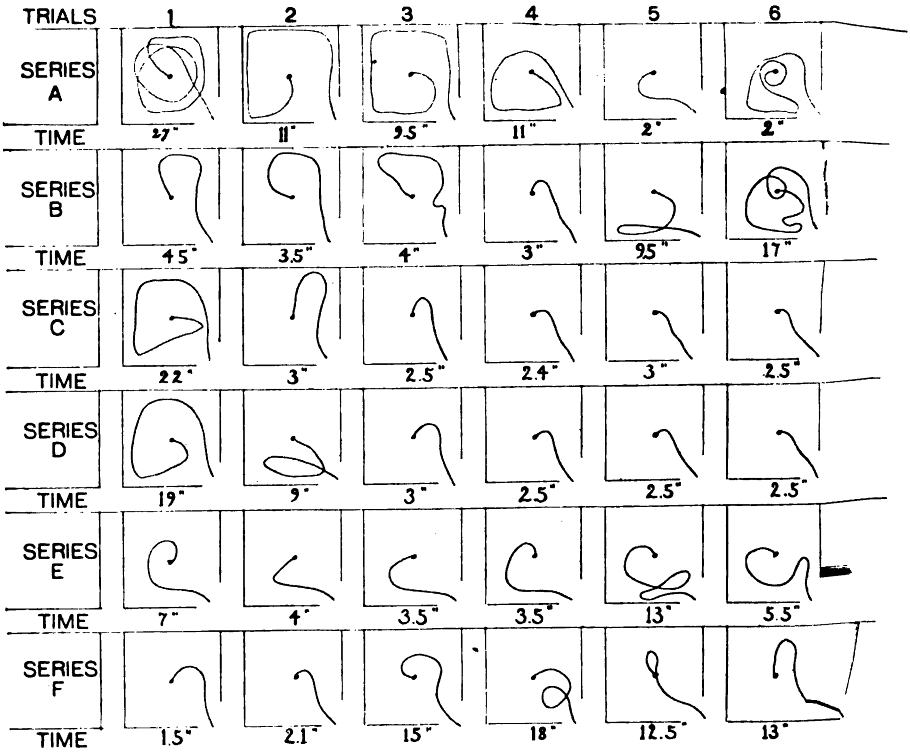

FIG. 2. Graphic representation of six sets of trials made by six individuals. The squares represent the tank, the incomplete corner, the open exit. Above the first series of records are figures indicating the number of each trial, whereas beneath the squares are the time records made in traversing the pathway indicated within each square. In every case the animal started from the central dot.

canals, or to inequalities in the right and left swimming musculature, or the neuro-muscular coördinations, or to a combination of these possible factors. Any one of them, or any combination, except of course a compensatory one, might be responsible for the fact that some animals naturally turn to the right, others to the left.

The determination of the true basis of this behavior would be very difficult because the repetition of the tests that bring it forth leads to the formation of a habit. That such becomes established, is, I think, sufficiently clear from the diagrams, as well as from the time records beneath them. Very interesting are series of which A is typical. In these, an animal, either because of its structure or its habit, turns consistently in one direction, in the present example, to the right. For some reason, exactly the reverse course was taken, with great success, at the fifth trial, and the sixth shows distinctly how this new departure complicated the course.

Series A illustrates a second fact, namely, that in many cases the pathway in succeeding trials becomes more complex, and the time consumed in escaping may increase. Nothing would be further from the truth than the conclusion that increase in either the complexity of the path or in the length of time, indicates progressive stupidity. The conditions of the experiments are such that a property analogous to cool-headedness in man, is at a premium. In many instances the animals became increasingly nervous, and lost their heads.

In problems whose solution can be effected, provided the animal has enough time and makes sufficient movements, this complication does not enter, but more than mere activity is necessary in a crisis. Animals which by luck or otherwise succeed early, behave much as they would in the face of "slow" problems; but animals that fail, or succeed only with difficulty, quickly become so handicapped by fear, and the useless activity characteristic of that state, that they fail more completely or succeed with much greater difficulty in later trials than they would in corresponding trials under more favorable conditions. While the experience of the tank brings out forcibly innate differences in the capacity to do the right thing quickly under stress, it is hardly adequate to measure real differences in the ability to learn. The individuals which are thrown out of commission, by the very nature of the problem itself, are simply out of the running; they are unable to compete, and their failure is no more to be ascribed to inferior intelligence than the failure of a blacksmith to win prizes at a swimming meet is to be ascribed to the superior strength of the other contestants.

Objectively, of course, the facts are that some animals fail, whereas others succeed. Success may come with progressive reduction in the complexity of the path, and in the time taken to traverse it, or with either of these elements separately; failure may result from the corresponding opposites. We can say with justice that the capacity to learn also expresses itself in one or more of these ways, but the obvious inference would not be the correct one. Should the chain of activities that we are interested in be inaugurated, our objective measurements would give us the information we want, but when some other chain is set up, the measurements show simply the extent to which the second disturbs the first chain. In other words, if the problems convert the animal into a non-learning mechanism, the movements which the individual performs throw no light on its learning capacity. What they do show is, that under the given circumstances, some animals improve, others do not, but the capacity of those that fail remains unknown. Even those that succeed do not divulge all. Strictly speaking, their capacity is shown to be not less than the records indicate, but actually it may be considerably more.

The animals that improve emphasize a practical question of some importance. There are plainly two ways of solving the tank problem; by increasing speed, and by decreasing the length of the pathway. Cases in which these two go hand in hand, or in which constancy of speed is offset by an abbreviation of the pathway, offer no difficulty; but what shall we say when increased complexity of the pathway is compensated for by heightened speed? If we limit ourselves entirely to the objective time measurements, such an individual may seem to improve or to hold its own; if, on the other hand, we study the pathway alone, the animal is clearly loosing in fitness. The sixth trial in series A is a concrete illustration. How shall such an individual be rated?

As the objective records are at variance, it seems at first sight arbitrary to fix on either one or the other, and to say this is a measure of the truth.

Series C and D are from animals naturally well fitted to cope with the problem presented; natural fitness, however, varies, and some individuals inaugurate an overpowering set of altogether irrelevant, interfering movements. Such animals, practically, are not competing. Between these extremes most of the other individuals take their places, for their irrelevancies are not sufficient to destroy all chances of success, though marked enough to affect the general averages. The problem is to get out of the tank as quickly as possible, and is solved both by the animal that reduces the complexity of its path, either with or without an increase of speed, and by the animal that compensates inferiority in one direction by superiority in another. The latter might even win, but if we were offering prizes, justice would demand one for improvement in speed, the other for improvement in form. As form in the end makes for speed, not in individual cases, but on the average, the time records may be adopted officially as a practical, though not necessarily complete, measure of fitness.

SPECIFIC RESULTS

ADULT RATS

The first experiments were made with a tank differing slightly in size from the one described, and the animals, instead of being dropped into the water through a shoot that landed them in the exact center, were thrown in from one side, with a slight whirling movement. There was but one corner opening through which escape might be effected. The problem was thus essentially the same as the one described, and the differences in the records are mainly due to differences in the sizes of the two tanks, and to the way in which the animals were introduced. The whirling movement was adopted in order to insure that the effort to escape might not be begun in any except a chance direction. The results are tabulated below in self-explanatory form.

ANIMAL I.			ANIMALS II.			ANIMAL III.		
Trials	Time	Intervals	Trials	Time	Intervals	Trials	Time	Intervals
1	19.0″	0	1	14.0″	0	1	6.0″	1′30″
2	3.5″	0	2	11.0″	0	2	9.0″	1′10″
3	2.0″	1′	3	9.0″	1′	3	8.4″	1′30″
4	5.0″	15′	4	5.0″	0	4	26.0″	2′03″
5	3.5″	0	5	2.0″	15′	5	1.4″	2′00″
6	2.5″		6	2.9″	0	6	1.0″	Hours
			7	17.0″		7	1.4″	1′32″
						8	1.2″	1′20″
						9	1.6″	2′40″
						10	2.4″	0′40″
						11	2.0″	0′45″
						12	1.0″	

Characterization, Animal I: At first many trial movements, then stopped investigating. In the end did not hurry out. Averages: first three trials, 8.16″; second three trials 3.66″. Improvement, 55.1 per cent.

Characterization, Animal II: Accidental delay at second trial. Numerous extra movements in fourth. In seventh gave up trying to get out, hence this record is omitted in the averages. Averages: first three trials, 11.33″; second three trials, 3.33″. Improvement, 70.8 per cent.

Characterization, Animal III: Slight delay in starting watch at eighth trial. Averages: first six trials, 4.73″; second six trials, 1.60″. Improvement, 66.3 per cent.

ANIMAL IV.			ANIMAL V.			ANIMAL VI.		
Trials	Time	Intervals	Trials	Time	Intervals	Trials	Time	Intervals
1	57.4″	30″	1	55.2″	30″	1	13.1″	30″
2	3.2″	30″	2	7.1″	30″	2	18.1″	30″
3	8.2″	30″	3	5.1″	30″	3	4.3″	30″
4	12.3″	30″	4	6.3″	30″	4	5.1″	30″
5	8.2″	30″	5	5.2″	30″	5	5.2″	30″
6	8.2″	20″	6	15.3″	20″	6	3.3″	20″
7	9.3″	30″	7	3.3″	30″	7	12.2″	30″
8	4.4″	30″	8	6.0″	30″	8	13.1″	30″
9	4.3″	30″	9	12.2″	30″	9	22.3″	30″
10	3.2″	30″	10	19.2″	30″	10	8.0″	30″
11	3.1″	30″	11	4.0″	30″	11	31.4″	30″
12	5.0″		12	4.0″		12	4.3″	

Characterization, Animal IV: Animal quiet. Averages: first six trials, 16.25″; second six trials, 4.88.″ Improvement, 70 per cent.

Characterization, Animal V: Animal quiet. Averages: first six trials, 15.7″; second six trials, 8.11.″ Improvement, 48.4 per cent.

Characterization, Animal VI: Animal quiet in the first six trials, and apparently improving at a good rate. In the second set, however, it suddenly became "cranky" and did everything except the expected; it swam around aimlessly, or clawed the covering screen. Averages: first six trials, 8.18″; second six trials, 15.20″. "Deterioration," 85.8 per cent. This record is of necessity omitted in the later calculations.

Naturally there is much unevenness in these records, and at times the irregularities seem to obscure the evidence that a habit was formed, or to show that the exact opposite was established. Nevertheless there is a fundamental harmony beneath the discrepancies, and this is not destroyed even when the record of animal VI is admitted. For obvious reasons, however, it would be unfair to allow this animal to figure in calculations that involve the group of animals as a whole. On similar grounds I have excluded the 7th trial of animal II, for on that particular occasion this individual also very clearly did not attempt to escape. If with these modifications the results be taken as they stand, we can construct the following table for comparisons:

	FIRST HALF RECORD Averages *Seconds*		SECOND HALF RECORD Averages *Seconds*
Animal I	8.16		3.66
Animal II	11.33		3.33
Animal III	4.73		1.60
Animal IV	16.25		4.88
Animal V	15.70		8.11
General average	11.23	General average	4.32

A glance at this record shows that experience, even when limited to very brief periods of intense activity, has its effect, and in the adults composing this group brought about on the average an increase of 6.89 seconds in speed, or an improvement of 62.1 per cent. Considering the unfavorable conditions for the formation of a habit which these experiments present, the result is very marked.

YOUNG RATS

That this conclusion is not a mistaken one, is indicated by a similar treatment of the records of very young animals that underwent the experience of the tank. The individuals whose performances are tabulated in detail below were all from the same litter, and were aged three and a half weeks.

ANIMAL A.			ANIMAL B.		
Trials	Time	Intervals	Trials	Time	Intervals
1	9.0″	1′25″	1	5.0″	15″
2	4.0″	44″	2	13″	37″
3	5.0″	19″	3	14″	27″
4	2.5″	43″	4	10″	25″
5	5.0″	28″	5	4.5″	30″
6	2.0″	45″	6	5.0″	31″
7	3.0″	23″	7	7.0″	1′2″
8	1.3″	45″	8	3.5″	1′9″
9	1.5″	1′10″	9	4.5″	1′8″
10	1.0″	1′15″	10	2.0″	58″
11	2.0″	56″	11	5.5″	32″
12	2.0″		12	6.0″	

Characterization, Animal A: Animal between three and four weeks old; quick in its movements and not easily confused. Took a new route at the fifth trial, but at the sixth headed at once in the proper direction. Averages: first six trials: 4.58″; second six trials, 1.8″. Improvement, 60.7 per cent.

Characterization, Animal B: Animal between three and four weeks old; quick but irregular and not to be counted on. In the twelfth trial ducked under just before emerging, and lost considerable time. Averages: first six trials, 8.56″; second six trials, 4.75″. Improvement, 44.6 per cent.

Taking the figures as they stand, and comparing the average of the first half-record of each animal with the average of the second half-record, we get the following:

	FIRST HALF RECORD Averages Seconds		SECOND HALF RECORD Averages Seconds
Animal A	4.58		1.80
Animal B	8.56		4.75
Animal C	13.75		4.80
Animal D	6.43		4.54
General average	8.33	General average	3.94

Here, too, is evidence that the behavior was modified by the "tank experience," but much more interesting is the fact that the changes undergone seem to harmonize very closely with the results secured by Watson ('03) under much more favorable conditions. The increase in the speed of the young animals was

ANIMAL C.			ANIMAL D.		
Trials	Time	Intervals	Trials	Time	Intervals
1	8.5″	42″	1	10.5″	24″
2	33.0″	22″	2	8.5″	35″
3	19.0″	20″	3	5.8″	1′10″
4	9.0″	34″	4	5.8″	40″
5	9.0″	36″	5	6.0″	47″
6	4.0″	53″	6	2.0″	56″
7	3.0″	1′	7	7.0″	40″
8	3.0″	1′4″	8	3.0″	53″
9	3.0″	43″	9	3.3″	1′10″
10	2.8″	1′7″	10	6.0″	58″
11	4.0″	1′28″	11	4.3″	42″
12	13.0″		12	3.5″	

Characterization, Animal C: Animal between three and four weeks old; very quick and nervous. In first trial explored all corners, became panic-stricken in the second, better in the third, but during the fourth lost much time clawing the wire screen. In the last half of the series the animal seemed to have found itself, and exhibited no semblance of fear. The length of the twelfth trial is due to having overshot the opening. Averages: first six trials, 13.75″; second six trials, 4.8″. Improvement, 64.4 per cent.

Characterization, Animal D: Animal between three and four weeks old. Relatively slow and indifferent. Averages: first six trials, 6.43″; second six trials, 4.57″. Improvement, 29.9 per cent.

on the average 4.39 seconds, for the adults 6.89 seconds; the former represents an increase of 53 per cent in efficiency, whereas the adults improved 62.1 per cent.

DURATION OF THE HABIT

That a habit formed under stress during brief periods of intense activity, may endure from fifteen minutes to several hours, the records already given show plainly. Special tests were made, however, to determine, if possible, whether any traces of the heightened efficiency might be found after several days. For this purpose, animals A, B, and C, of the preceding group of young were used, and adults A, B, C, D, E, and F, upon whose records fig. 2 is based. In the present connection I shall distinguish the three young animals by small letters, the six adults by means of capitals.

ANIMAL	DATE	TIME	TRIALS	AVERAGES
				Seconds
a	4/26	2.15 p.m.	6	4.58
a	4/26	3.28 p.m.	6	1.34
a	4/27	2.15 p.m.	6	4.69
b	4/26	2.30 p.m.	6	5.15
b	4/26	3.35 p.m.	6	4.75
b	4/27	2.30 p.m.	6	3.30
b	5/9	10.20 a.m.	6	5.65
b	5/9	10.45 a.m.	6	2.10
c	4/26	2.50 p.m.	6	13.40
c	4/26	3.43 p.m.	6	4.79
c	4/27	2.50 p.m.	6	7.00

The adults A, B, C, D, E, and F, whose records on January 15th, 20th, 22d, and 28th follow, were all used prior to December 11th of the year before, but unfortunately the earlier experiments were not performed under exactly the same conditions as those made in January, and a comparison of records separated in time by more than a month cannot be made. The results of the series separated in time by five days, by two, and again by six, can, however, be safely compared.

ANIMAL	DATE	DATE	DATE	DATE
	Jan. 15	Jan. 20	Jan. 22	Jan. 28
	Seconds	*Seconds*	*Seconds*	*Seconds*
A	10.40	1.66	1.91	2.08
B	6.90	10.25	5.83	6.00
C	5.90	9.08	13.75	10.50
D	6.41	5.66	6.08	6.08
E	6.08	32.82	11.41	9.75
F	15.75	19.00	16.00	10.16
General average	8.58	13.08	9.16	7.33

The results from the young animals are not especially conclusive. Taking the records as they stand, however, it may be said that two out of the three seemed to show the effects of their experiences twenty-four hours afterward, and one of the animals, after twelve days, very quickly recovered and in the end actually bettered its previous best record. It is to be expected, of course, that individuals vary greatly in the length of time which their habits, whether formed slowly or rapidly, endure; and furthermore, there is no way in which one can be reasonably certain, except by the method of multiple instances, whether, in the long run, animals without previous experience might not, on the whole, make as good records as those made by experienced individuals. As far as the group as a whole is concerned, it does not seem to bear markedly one way or the other on this particular question, although it does show that the final records of animals b and c were better than their initial ones.

Considering the adults, not as individuals, but as a group, and comparing the averages of the four series, we get the results as tabulated. Individual differences notwithstanding, we may say that, on the whole, the second set of trials, five days after the first, was distinctly slower; the third, two days after the second, not as slow as the preceding, whereas the fourth, six days after the third, was the best of all. Of course, these statements have no bearing on specific cases. The record as given is sufficiently detailed to show how the relative fixity in one individual is balanced, and even discounted by the relative instability of another, or vice versa.

INTRODUCTION OF ADDITIONAL STIMULI

The "tank experience" may be complicated by the introduction of additional factors, which, classified by their effects, may be called retarding and accelerating stimuli. Marked accelerations were produced by allowing the animals before reaching the water in the tank, to fall through a paper bag filled with water considerably warmer; also if, during their fall through the cylinder, the animals received an electric shock, the speed was increased. On the other hand, the addition of salt in small quantities to the water in the tank had, on the average, a depressing effect. The most marked, as well as constant effects, however, were gotten by the system of desired rewards. Animals that had been without food for twenty-four hours, were dropped into disagreeably cold water, and immediately on escaping, were wrapped in warm towels and given a nibble of cheese. All these factors helped to make escape from the tank better worth while than it had been in any of the preceding experiments. The results, based on two full-sized adults, and on eight young, less than a year old, are tabulated below. As heretofore, the adults are labelled with capitals, the young with small letters.

ANIMAL	FIRST HALF		SECOND HALF		IMPROVEMENT
	Record	Time	Record	Time	
	Trials	*Seconds*	*Trials*	*Seconds*	*Per Cent*
AA	6	7.45	6	3.71	51
BB	3	15.5	3	8.2	48
aa	6	10.8	6	3.2	69
bb	6	13.03	6	5.26	60
cc	6	9.8	6	7.3	26
dd	6	16.65	6	12.66	24
ee	6	13.3	6	7.2	46
ff	6	15.9	6	6.3	61
gg	6	18.8	6	3.2	83
hh	6	8.3	6	3.7	56

Not only are the adults too few, but the number of trials made is too small to allow of satisfactory comparison with the earlier records made under simpler conditions. Neither AA nor BB improved as much as the adults in former experiments, but this may have been because they were unusually slow. If a conclusion may be hazarded, it is that the additional stimuli had no effect or perhaps a slightly depressing one, though this is uncertain.

The young, on the other hand, improved on the average 62.5 per cent, as compared with an earlier record of 53 per cent. Animals cc and dd have been eliminated from these calculations on the ground of over-excitedness. The results might have been expected. There was an unmistakable effect on the young, and in two cases the additional discomforts and rewards produced so much activity, that these particular records were vitiated by it.

THE SENSES AND THE HABIT

The relation between the senses and the acquisition of habits in the white rat has been so thoroughly worked over by Watson ('07) that I have performed only a few experiments, the results of which I shall present for two reasons, first because they are corroborative, in spite of the differences in method; secondly, because they show that the various senses can be eliminated without resorting to the extirpation, or destruction of the sense organs. Under the conditions of the experiments, hearing, smell, sight, and touch, either singly or in any combination, may conceivably furnish the sensual basis on which the habit rests.

HEARING

As the associations were formed while one of the four exits was open, it may have been that the movements of the animals were influenced by the differences in sound intensity or quality due to the unblocked passage. Such differences, if they exist, must be very small, for the closed openings are blocked simply by wire netting, but as rats are known to perceive minute sound differences, the point was well worth testing.

Associations were established in the usual way, after which the exit to which the animal had been trained, was closed and one or several of the others were opened. In every case the individuals continued to go to the exit from which they had previously escaped. Of course this test could not be continued indefinitely, as failure to escape from the tank, resulted in new exploratory movements that in the end led the animals to one of the other openings. Frequent visits to the old corner are characteristic of these secondary trials.

In another experiment, the association was established amid general noise, so great just above the center of the tank, that probably there were no differences at any of the corners. When the animals, after a brief rest, were placed again in the tank, they were allowed to make their second series of trials in absolute silence. In all cases they swam to the right place with no more variation in time or in the complexity of the pathway than is found under ordinary conditions.

A third test was made by allowing the association to establish itself while a noise was made at one of the other opened escapes. These noises, purposely not loud enough to frighten the animals, but certainly marked enough to be audible, seemed to have no effect whatever.

SMELL

To determine the part played by the sense of smell, the exit left open was thoroughly perfumed with cheese. After the association was established, the tank was turned in such a way that the perfumed opening was diagonally opposite its original position, whereas another exit, unperfumed, occupied geographically the place previously held by the escape to which the animal had been trained. Variations of this plan, such as holding a piece of cheese, first in one corner, then in another, were also tried, but in each case the animals swam in the direction that led to the earlier escapes. So far as these tests of the importance of smell are concerned, they give only negative results.

SIGHT

The experiments on sight bear simply on such rays as we ourselves are able to perceive, and were performed in a carefully constructed and thoroughly efficient photographic dark room. From the subjective standpoint, the animals were allowed to swim in the dark, but whether what we call dark is in reality dark to a rat, is another question.

Two sets of animals were used, two adults, and two young ones less than a year old. Each animal was given four sets of six trials. The averages follow:

ANIMAL	DATE	TRIALS	AVERAGES
			Seconds
A	11/11	6	11.03
A	11/11	6	17.10
A	11/12	6	16.40
A	11/13	6	12.73
B	11/12	6	13.40
B	11/12	6	24.96
B	11/13	6	6.42
B	11/13	6	11.76
a	11/11	6	18.23
a	11/12	6	7.05
a	11/12	6	6.40
a	11/13	6	2.00
b	11/13	6	96.00
b	11/13	6	50.24
b	11/14	6	31.56
b	11/14	6	10.90

If the first two series of A are compared with the last two, it will be found that this animal remained practically constant in speed; if a similar comparison be made for B, we find that this individual increased its speed on the average by 9.28″; whereas the records of a and b show increases in efficiency in each of the last three sets. Undoubtedly, then, the tank problem can be solved in the dark, and the solution, may with repetition, become a habit, but the evidence does not show that the sense of sight plays no part under other circumstances. Watson's negative evidence on this point seems very good, but the maze problem and the tank problem differ so much, that what is true in the solution of the one, is not of necessity true in the solution of the other.

TOUCH

Touch in the ordinary sense, is practically eliminated by the nature of the problem. The actual solution takes place in a medium in which the animals float. There are no solid bodies to be touched; no differences in homogenity in any direction, that might guide the individuals to the correct opening. The same thing applies to the temperature sense. It is possible, however, that touch and temperature, singly or together, affect the result, but if they do, it is not in the usual way, but as the initiating stimuli of other activities.

THE KINÆSTHETIC CHAIN

With hearing, smell, sight and touch, either eliminated, or shown to be not essential, the question of how the habit becomes established, naturally arises, Watson ('07) and others have presented a large body of evidence suggesting that the kinæsthetic sense may be the controlling factor in the behavior of the white rat. My own experiments seem to bear similar interpretation, but if the facts corroborated by the tank method are due to a kinæsthetic sense, it follows that this needs investigation.

Granted that a relation exists between the objective phenomena which we call a habit, and the inferred basal kinæsthesia, the inference that the relation is definite, seems just; for the habit itself is definite, and capable not only of exact measurement, but also of modification. It follows that in the emergence of a habit, the internal basis also is modified, for the habit itself is modified behavior. In other words, kinæsthesia is easier to understand if thought of as a sequence of states which will be repeated, provided the physiological state of the animal remains favorable, whenever the stimuli that started it in the first place recur.

It is here that any sensations associated with the beginning of a solution may come into play. Unfortunately, I have no results to offer on this subject at present, but the standpoint itself may not be without value. If kinæsthesia is indeed a special element in the sum total of internal conditions, is in fact a chain, based on the physiological, and expressed objectively in the habit, the answer to a difficult problem may be a little easier to find than heretofore.

SUMMARY AND CONCLUSIONS

My purposes in presenting these results have been to show the adequacy of the tank as an instrument for studying animal behavior; to show that our usual methods leave out of consideration a large range of interesting activities; and finally to demonstrate that behavior, limited to brief periods of very intense exertion under stress, may lead to the formation of habits.

Incidentally, the results seem to corroborate, as far as they go, some of the conclusions set forth in Watson's splendid work, and wherever at variance, are so probably from the differences between the problem of the maze, and the problem of the tank. In certain ways, the latter seems well suited for studying the "direction sense" or whatever it is that in the absence of sight, hearing, smell, and touch, enables the animals, not only to solve the problem, but to improve in efficiency. If kinæsthesia plays an important role, if indeed it is by this means that the animals sense direction, and if furthermore, it is a sequence of states, dependent on the physiological condition of the animal, and on certain initiating stimuli, then by variations in the last two categories, one should get changes in kinæsthesia, which in turn would be registered by corresponding differences in the objective habit.

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Zoölogical Laboratory, University of Michigan.
January 13th, 1910.

PAMPHLET COLLECTION

THE NEMATOCYSTS OF EOLIDS

By
OTTO C. GLASER

ELEVEN FIGURES

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THE NEMATOCYSTS OF EOLIDS[1]

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ELEVEN FIGURES

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INTRODUCTION

The note on the nematocysts of nudibranch molluscs which I published in the Circular of the Johns Hopkins University in March, 1903, was the outcome of suggestions made by Professor Brooks, not only as to the possible origin of eolidian nettles, but as to the advisability of presenting at once the evidence at that time available. The necessity of teaching in summer schools during the only months when work on eolids is possible has caused the delays which have robbed me of the pleasure of offering my results prior to partial anticipation by others, and in time for Dr. Brooks to see them.

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[1] From the Zoölogical Laboratory of the University of Michigan.

In addition to the rhinophores (fig. 1), with their parallel rings, and the mobile foot-tentacles, sensitive to touch, eolids possess numerous dorsal appendages, or cerata. These structures, often banded distally, and tipped with blue, purple, grey, or white, occur in clusters symmetrically distributed on the sides of the body. When, as is frequently true, the cerata are very abundant, their distribution seems to be uniform; nevertheless each ceras belongs to a particular group that radiates from an enlargement on the side of the body. These swellings are regions of proliferation and from them arise new appendages in such manner that the oldest in each collection is nearest the sagittal axis of the animal.

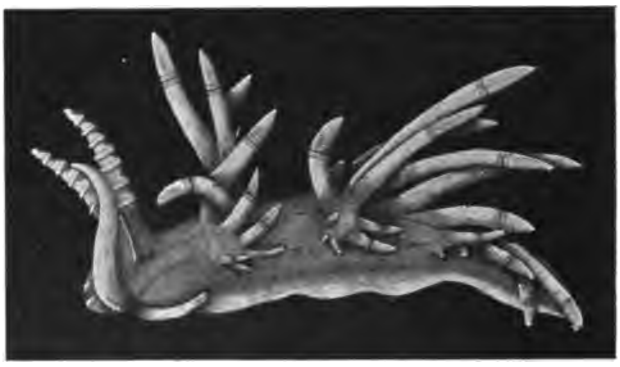

Fig. 1 Typical eolid, showing rhinophores with parallel ring-like swellings; foot-tentacles, and cerata of various sizes radiating in groups from lateral enlargements.

A median section through such a ceras (fig. 2), shows an ectodermal covering (*ECT*), indistinct in cell-boundaries, and rich in mucous glands. Inside this are bundles of longitudinal and circular muscles (*MUSC*), enclosing a more or less capacious duct whose distal pore (*CNDP*), completes a passageway from the lumen of the liver to the outside. This duct, lined proximally with hepatic cells, then with ciliated epithelium, leads distally into a cnidophore filled partially or completely with cells or cysts containing nettles. The basal entrance into the cnidophore is guarded by a sphincter (*SPH*).

The morphology of the cerata is so thoroughly understood, that there is no need to add anything to the writings of Davenport ('93), Hecht ('96), or Krembzow ('02). The origin of the nematocysts, however, is still a subject for debate since many biologists remain incredulous despite the published evidence. Grosvenor ('03) pointed out that this was the attitude assumed by some authorities toward T. Strethill Wright, who in 1858 read, before the Royal Physical Society of Edinburgh, a paper in which he attempted to show that the nematocysts of eolids are

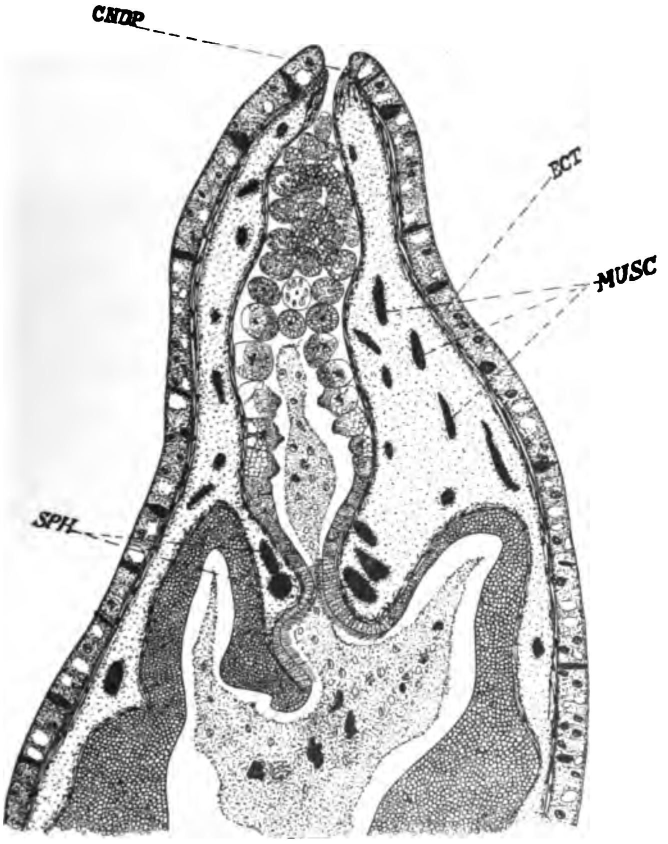

Fig. 2 Diagrammatic median longitudinal section through ceras showing complete cnidophoral system. CNDP., cnidopore; ECT., ectoderm; MUSC., longitudinal and circular muscles; SPH., sphincter.

derived from their hydroid prey. According to Wright, the possibility of this had occurred previously to Huxley and to Gosse, the latter of whom suggested methods of experimentation. Wright's evidence failed to convince Alder who considered the extraneous origin of the nettles improbable. Bergh, too, in a paper, abstracted in the Microscopical Journal ('62), described the nematocysts of several additional genera and species and interpreted them as secretions of the cnidophore.

Bergh's failure to discuss Wright's work, led the latter to publish in the next volume of the same journal an abstract of his former paper, together with additional evidence which seemed to confirm the original contention. Grosvenor says: "Owing either to the absence of figures or the aforementioned improbability of the conclusions it contained, this abstract seems to have been overlooked as completely as the original paper." All observers, from 1861 to 1903, failed to acquaint themselves with Wright's work, and assumed that the nematocysts were "manufactured" by the eolids in which they are found. Bergh himself, however, seems afterward to have entertained suspicions.

In the autumn of 1903 G. H. Grosvenor resurrected Wright's results, which, like all other writers, I too had overlooked, and on the basis of new experiments and histological observations gave an excellent account of the origin of the nematocysts. Recently Cuénot ('07), doubting both Grosvenor's statements and mine, re-investigated the subject, and now believes that the nettles are derived from cœlenterates. Cuénot's paper, however, contains fewer cytological details than the earlier one by Grosvenor.

EVIDENCE BEARING ON THE ORIGIN OF THE NEMATOCYSTS

One who maintains that the nematocysts of eolids are derived from their cœlenterate prey, must be prepared to answer certain questions:

a. Do eolids feed on cœlenterates?

b. Are the nematocysts of eolids identical with those of their prey?

c. Are nematocysts indigestible?

d. Can the nematocyst content of an eolid be altered by a change in diet?

e. Does a nematocyst-bearing species ever have individuals devoid of nettles?

f. Can the transfer of nematocysts from cœlenterates to the cnidophores of eolids be followed in detail?

The diet of eolids

The literature is full of testimony alleging the close association of eolids and cœlenterates. Moreover, eolids have frequently been observed while feeding on their cœlenterate neighbors. I have often seen them browsing on Tubularia crocea; on Eudendrium racemosum; and on actinians. Many species are so translucent that their food gives its color to the entire animal, and individuals which have fed on the pink heads of Eudendrium, or the somewhat paler ones of Tubularia, have the exact tints characteristic of these hydroids. Careful examination shows that the colors are due to undigested food in the alimentary canal and its diverticula.

Identity of eolidian and cœlenterate nettles

As Grosvenor says, "Those who quote the nematocysts of nudibranchs and cœlenterates as a striking example of homoplasy or convergence, can scarcely be aware of the astonishing completeness of this assumed convergence." His assertion is supported by a considerable number of cases in which the isomorphism extends to the minutest details. Wright and Cuénot made similar observations. A number of equally striking instances have come to my notice, but I shall describe only two.

Among the sagartias attached in great numbers to oyster shells, at Beaufort, N. C., I found one eolid with nematocysts absolutely like those occurring in the tentacles of the actinian. The undischarged nettle of sagartia is a small rod in whose long axis lies a straight filament. Unexploded capsules answering to this description were found in the cnidophores of the eolid (fig. 3, *A*). The complete identity of the two kinds was demonstrated by the discharged filaments, for these are characteristically barbed. Near its base (fig. 3, *B*), each thread has a large number of minute barbules directed toward the capsule. This region is followed by one with stouter barbs, and this in turn by one with small. The extreme tip of the thread is bare. Such a filament is sufficiently marked to make its recognition easy.

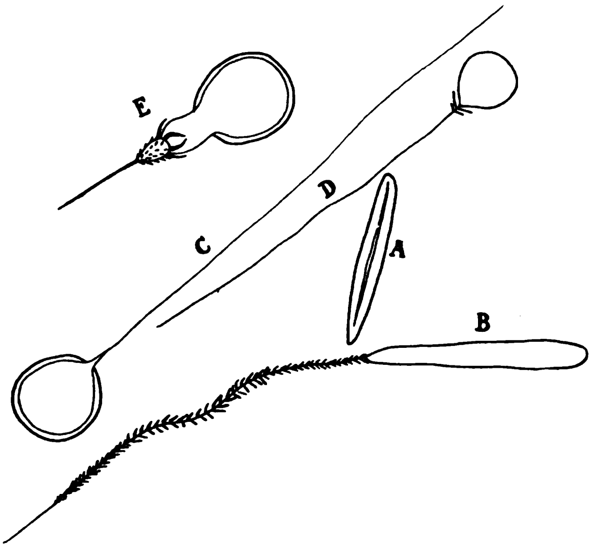

Fig. 3 **A, B,** nematocysts of Sagartia and of an eolid which preys on Sagartia; **C, D, E,** three types of tubularian nematocysts found in eolids which prey on Tubularia.

The most striking case which I have observed is the following: at Beaufort, also, another species of Eolis lives on Tubularia. An examination of the undischarged nematocysts of this tubularian divulges no great differences among them, but when exploded three kinds are easily recognizable. One (fig. 3, *C*), has a long delicate thread completely devoid of barbules, and widening near the base where it is continuous with an almost spherical capsule. A second type (fig. 3, *D*), less frequent, practically duplicates the first, except in two particulars: the capsule is considerably smaller, and the thread instead of widening at its base, is provided with two pairs of barbs, of which the stouter is about three times the length of the other. The third type of nematocyst (fig. 3, *E*) is the most common. In this the capsule is ovoid, and the discharged filament presents distinct regions. At the place where evagination occurs there is a bare projection crowned by four stout barbs with points directed toward the capsule. These four barbs originate in a crease between the barren mound and a considerably smaller distal swelling with many minute barbules pointing, like the large ones, toward the capsule. From the tip of this barbulated swelling the undifferentiated portion of the thread arises. The thread itself, however, is not without distinguishing characteristics; it is about twice the thickness of the threads of the first and second type, and about one-fifth as long; it is devoid of barbules and ends in an excessively fine point.

The cnidophores of eolids which prey on Tubularia contain nematocysts which in their discharged state agree point for point with one or the other of the three types found in the hydroid. That these three types represent developmental stages of only one kind of nettle seems unlikely, but even if they do, the argument from identity remains unchanged.

Indigestibility of nematocysts

If the source of eolidian nettles is cœlenterate food, it follows that the latter is not completely digestible. In a paper on the physiology of nematocysts ('09), I described certain methods of isolation whose success depends on the immunity of the capsules to at least two forms of digestion, peptic and putrefactive. In order to discover whether the nematocysts of eolids could be treated without destruction in the same manner as those of Hydra, Metridium, and Physalia, I subjected a large number of the cerata of Montagua to peptic digestion. The experiment gave a positive answer. Without immunity to digestive enzymes eolidian nettles could not be derived from cœlenterates, but the fact that they are indigestible is no argument in favor of derivation, since in the opposite case they would probably be equally resistant.

Change of diet

Wright, Grosvenor and Cuénot, have studied the effect of an altered diet on the nematocyst content of eolids, and have demonstrated conclusively that the nettles vary with the food. Grosvenor's results particularly are so striking that there is scarcely need of insisting further on this point. I shall, however, add one case out of a number which I have collected.

In attempting to change the natural food of an eolid, one is confronted with an instance of strikingly specific diet, a condition suggested in nature. Montagua for instance, occurs only on Tubularia crocea, even when other hydroids are present. Indeed, late in the summer, when the colonies of T. crocea are degenerating or have almost disappeared, while Eudendrium is in fair condition, and Pennaria is flourishing, Montagua does not change its diet; instead it clings to its food supply as long as this endures, and in the end disappears to return only with the next crop of the hydroid.

This pronounced specificity interferes with attempts to replace the natural food of a species, but in a few instances I was able to substitute Aiptasia for Tubularia. The cerata of an eolid treated in this manner contained afterward the nematocysts characteristic of the anemone. A section through such a ceras is copied in fig. 4, and shows in addition to the great number of naturally captured nettles, those artificially introduced (*A-F*).

Eolids devoid of nematocysts

Grosvenor has cited Calma glaucoides as an eolid normally devoid of nematocysts. According to Hecht, C. glaucoides feeds on the eggs and embryos of Cotta and other fish. Whereas this species has neither nettles nor cnidophores, Calma cavolinii, the only other member of the genus, feeds on hydroids and has typical cnidophores crowded with nematocysts.

These very suggestive facts can be duplicated within a given species. Thus in an earlier paper ('03), I mentioned an eolid which had been captured accidentally either before or during its

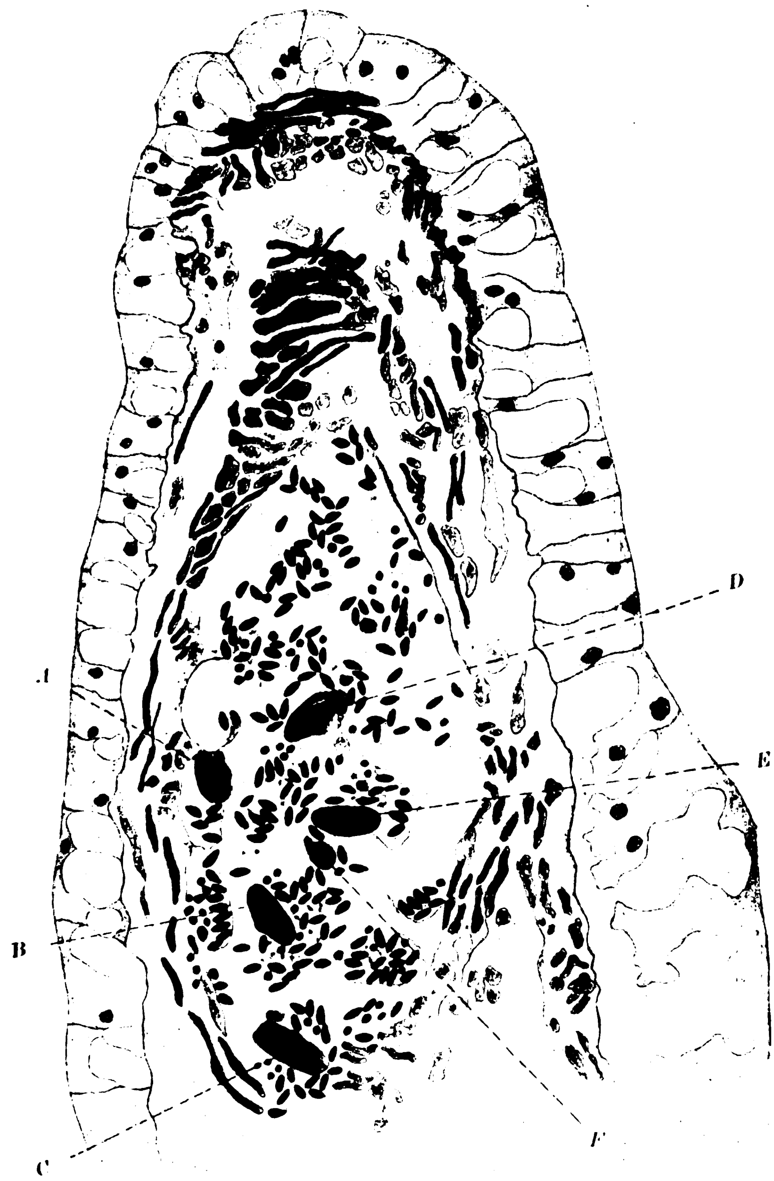

Fig. 4 Longitudinal section through the ceras of an eolid showing the normal nettles in great profusion, and at *A*, *B*, *C*, *D*, *E*, *F*, the artificially introduced nematocysts of Aiptasia. Drawn by Mr. Carl Kellner.

metamorphosis, and had completed that process in an aquarium in which it lived for over two months. During the early part of this period, a few hydromedusæ were present, but these soon died out, and the eolid passed the greater portion of its captivity in the absence of cœlenterate food. Serial sections, upon careful examination, since repeated, revealed nothing that could be identified as a nematocyst, either in the liver or in the cnidophores.

Even under natural conditions cases of this kind may occur. Like Krembzow, I have found, on several occasions, eolids practically devoid of nematocysts, as well as barren cnidophores among many heavily charged. These facts are easily understood if the nematocysts are derived from cœlenterates, but on the contrary view they appear as abnormalities without known explanation.

The experiment of hatching and rearing the young in aquaria free of cœlenterates, would give an unequivocal answer could it be carried out. Many difficulties however, present themselves, most important of which are the fatal attacks of the infusorians inevitably introduced with the food and water. Cuénot ('07), nevertheless was able to make tests of a strictly analogous nature. By clipping the cerata and allowing regeneration to occur in the absence of cœlenterates, he produced eolids with appendages partly free of nettles. The failure to regenerate cerata entirely devoid of nematocysts was due to the presence of capsules in other portions of the digestive tract, in the lumen of the liver as well as of its diverticula, and engulfed even in the liver cells themselves. Many of these nettles found their way into the regenerating cnidophores, but even so, not in sufficient number to destroy the great contrast between the restored and the amputated appendages. This slight uncertainty in Cuénot's results could be eliminated entirely by starving the animals for four or five days before operating.

The transfer of nematocysts

Cuénot has said that the best evidence of the derivation of solidian nettles is their history. This has been most carefully worked out by Grosvenor, and certain points in it have been touched upon by Cuénot. In what follows, Grosvenor's account is substantiated in all important respects.

What happens during artificial digestion unquestionably occurs in the alimentary canal of an eolid. The tissues of the food cœlenterate are dissolved and the nematocysts remain in great quantities free in the lumen of the tube. Many of them are voided with the fæces, others find their way into the cnidophores.

The passage from the digestive tract proper to the cnidophore is through the ciliated canal. Even were there no direct observations such as those of Hancock and Embleton ('46), Trinchese ('77-'81), and Grosvenor ('03), the occurrence of the same kinds in both cavities which the canal connects would suggest the derivation of the one group of nettles from the other. More suggestive still is the presence of "foreign" bodies in the cnidophore, a fact noted by Hecht, by Hancock and Embleton, and by Grosvenor. I have found uninterpretable fragments of tissue, bodies not tissue-like, and diatoms, in the liver diverticula and in the cnidophores. The ciliated canal apparently has no power to distinguish

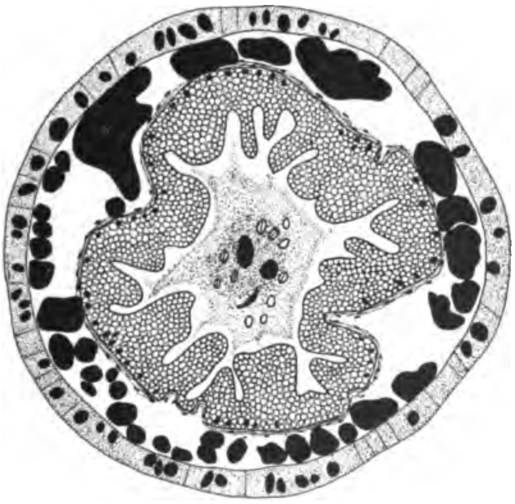

Fig. 5 Transverse section through the base of a ceras showing within the lumen of the liver diverticulum, certain uninterpretable fragments; a considerable number of undischarged nematocysts, and one crescentic diatom.

other indigestible bodies from nematocysts, but this inability to select is not shared by the cnidophages, for so far as I know, these ingest only nematocysts.

The cnidophages are derived from a zone of "embryonic" cells, located just distally to the ciliated canal. Fig. 6, a drawing of the proximal portion of a longitudinal section which happened not to strike the canal, shows the embryonic zone (*EMB. Z.*). Its cells have either no boundaries or incomplete ones; the nuclei are large and contain coarse chromatin granules; and the cytoplasm is undifferentiated. From this region of proliferation the oldest cells are pushed upward and enter the zone of cnidophages (*CNDPH.*) where, after ingesting a number of nettles inversely variable with the size of the engulfed capsules, they become converted into cnidocysts (*CNDCYS.*).

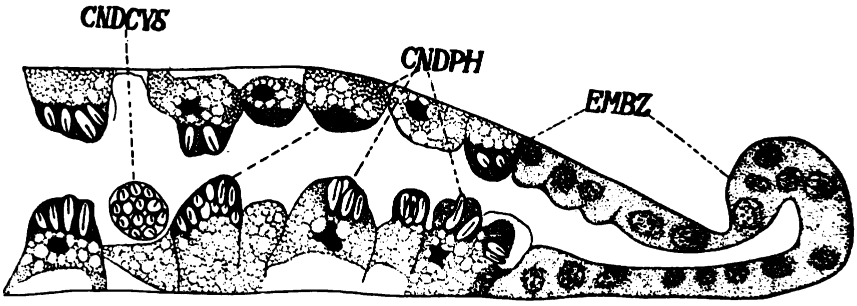

Fig. 6 Median longitudinal section through ceras; EMBZ., embryonic zone; CNDPH., cnidophages; CNDCYS., cnidocysts.

No single description of the cnidophore would prove satisfactory since the various zones that compose it present phases which depend in part on the age of the appendage, in part on the number of nematocysts ingested. In cerata in which "feeding" is actively going on, or in which it might go on, certain cells show evidence of pseudopodial activity. In the transverse section, fig. 7, the lumen of the cnidophore is traversed and rendered labyrinthine basally, by projections from its bounding cells. Processes from several cells may fuse into larger bridges with, at times, branch connections to other cells. Though visible only in sections, the projections suggest activity, as if they had been fixed in the act of moving, or flowing. It is interesting that cnidophores well-stocked, do not contain these labyrinths. Apparently if the "fishing" results in a "catch," the cells withdraw their pseudopods.

Not all the cells produced by the embryonic zone become cnidophages. In addition to these, certain interstitial cells are formed whose fate is quite different. Fig. 8 illustrates part of a longitudinal section through basal cnidophages. The cells have ingested no nematocysts, but those which were about to, differ distinctly from the prospective interstitial cells. The latter are very narrow, have near their bases small densely staining undifferentiated nuclei, and have cytoplasm, which, wherever visible, resembles that found in the embryonic cells. Near the lumen of the cnidophore the interstitial cells have no membranous boundaries, although elsewhere they are definitely delimited.

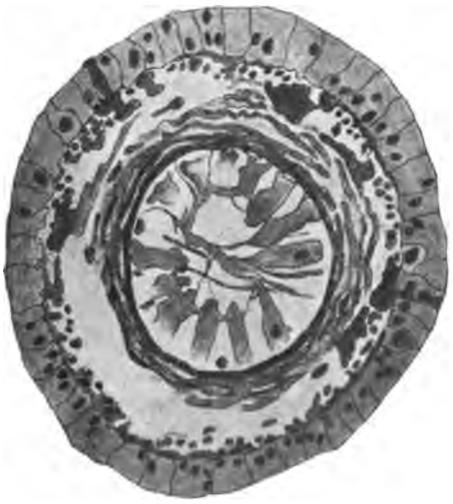

Fig. 7 Transverse section through a ceras in which the lumen is made labyrinthine by pseudopodia that project inwards from the bounding cnidophages. Drawn by Mr. Carl Kellner.

The prospective cnidophages undergo striking changes which emphasize still more the disparity in size between them and the interstitial cells. The young cnidophage enlarges, partly by the development of great vacuoles in the cytoplasm, (fig. 8), and partly because the cell contents withdraw from all except certain portions of their boundary, remaining connected with it only here and there by strands.

When the differentiation of the cnidophage is complete, it presents the appearance shown in fig. 9. Each cell has a receptive eminence in which the cytoplasm remains naked and in the embryonic condition. From this point pseudopodia may be sent out, but in appendages in which ingestion has taken place, only short, blunt processes are found.

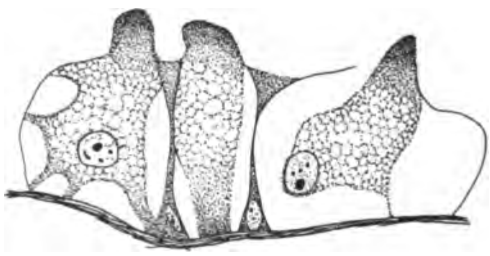

Fig. 8 Showing young cnidophages with blunt pseudopodia. Also interstitial cells.

After the cnidophage has ingested its fill of nettles, degenerative metamorphosis sets in. This process is characterized chiefly by retrograde changes in the nucleus, which gradually looses all visible differentiation, and becomes irregularly lobed or stellate (fig. 6). As the finished cnidocyst shows no histological details other than the nematocysts and their enclosing membrane, the inference that nucleus and cytoplasm both degenerate completely seems well founded.

The complete history of the cnidocyst remains in doubt. Grosvenor was not able to determine with certainty where the bounding membrane comes from, nor have my own efforts to solve the problem met with better success. A suggestion is all that I can offer. The wall of the cyst is tough, and has considerable thickness. It is apparently formed during the period when the cnidophage is degenerating. Under the circumstances it is not unlikely that this cell has nothing whatever to do with the formation of the cyst, but that the membrane in question is traceable to other cells. Grosvenor indeed suggested that the interstitial cells might aid in the secretion of the cyst; to me it seems equally plausible to assume that these cells alone are responsible. The best evidence which supports this view is fig. 10, in which are

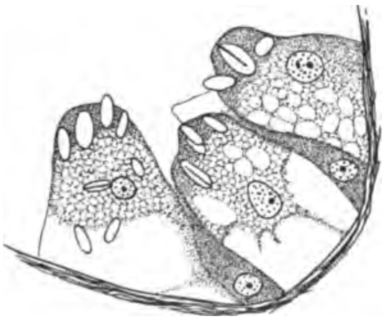

Fig. 9 Ingesting cnidophages showing differentiation and shrinkage from cell membranes. Also interstitial cells.

shown degenerating cnidophages, enclosed within compartments formed by interstitial cells which themselves have lost practically all of their original characteristics. As the figure illustrates they are reduced to mere membranes. Since each nettle-sac is complete, and in the ripe state capable of isolation from its fellows we must suppose on this view of the matter that the interstitial cell which lies between two or even three cnidophages contributes an independent portion to as many cnidocysts.

However they originate, the cnidocysts in their finished form are mere bags, thin-walled and transparent, filled with undischarged nettles. The cysts may lie free in the lumen of the cnidophore, or loosely attached to its walls. In either case they are easily extruded through the cnidophore by contractions of the cnidophoral musculature.

DISCUSSION

While the demonstrated identity of eolidian and cœlenterat nettles is clearly not due to homoplasy, the actual occurrences ar not so simply disposed of. Instinctively one wonders how suc complex interrelationships between two totally distinct sets organisms could have come about, and to what extent the pr cesses described may be said to be adaptive. Before attempting to deal with the questions that arise, it will be well to summarize briefly what can be easily understood if the collected evidence is valid.

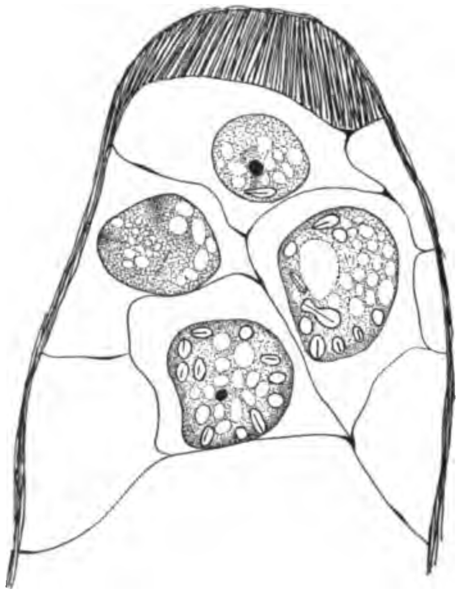

Fig. 10 Tangential section through the cnidophore showing young cnidocysts enclosed in spaces limited by degenerating interstitial cells.

If the accounts of Wright, Grosvenor, Cuénot and myself, are to be trusted, it is easy to see why the nematocysts should be found inside of entodermal cells and their derivatives; it is easy also to understand why, as has been pointed out by Krembzow, the nucleus of the cnidophage takes no part whatever in the "manufacture" of the nematocysts; further the occurrence of individuals and of cerata devoid of nettles is readily explicable; and finally the identity of eolidian and cœlenterate nettles is no longer a case of convergence that exceeds the probabilities of homoplastic evolution. However, some things remain to be explained.

The first requisite for the development of these remarkable relations, is that eolids shall be immune to the nettling organs of cœlenterates. Since nudibranchs are animals of unusual delicacy and apparently without protection, the freedom with which they crawl over hydroids and actinians, and browse upon their heavily charged tentacles, is intelligible only on the assumption that the dangers in the midst of which they live do not apply to them. It is not without interest to inquire how this can be.

The thought that most naturally comes to one is that in the course of time immunity has developed either by the elimination of individuals most prone to succumb to bombardment from nematocysts, or that in each generation the individuals, by being constantly under fire, gradually become indifferent to the punctures and stings, and finally fail entirely to react to stimuli to which the uninitiated respond by lively movements, and possibly by sensations of pain. This reasoning, however, rests on the assumption not only that eolids are vulnerable, but that they are under fire, and both premises require qualification.

That eolids are sensitive to the bombardment of nematocysts can be shown by transferring an animal which normally lives on Tubularia to the disc of a large Aiptasia. If the animal is not swallowed, it may fall off and reach the bottom of the dish in a perfectly rigid paralytic state. Occasionally spasmodic and angular movements may be observed, but recovery is rare as the animal usually dies despite many precautions.

If the discrepancy in size be reversed, so that the eolid is considerably larger than the Aiptasia, the latter seems to affect to a marked degree only the highly sensitive foot-tentacles, and the eolid under certain circumstances may devour the actinian. The attack is made at the base, and as the anemone immediately retracts its disc when assaulted, it is devoured in its least dang state. The outcome of the two experiments is thus very diff

It follows from this that even an absolute immunity to the n tocysts of one cœlenterate does not insure immunity aga those of another. The absolute immunity itself, however, c not exist. It has been said by several writers that eolids show evidence of having been punctured either from without or with by the sort of nettles in which they normally traffic are unknown Fig. 11 represents a cross section through a ceras taken from an

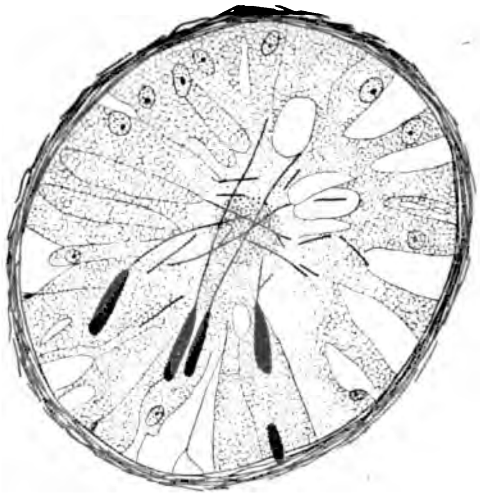

Fig. 11 Cross section through the base of a cnidophore in which intra-ceratal explosions have taken place.

eolid feeding normally on Aiptasia. The particular section is b no means representative, and gives a very inadequate idea of t number of discharges which has occurred in this appenda My reason for choosing it is the ease with which the individu filaments can be traced to their capsules. Other sections conta inextricable snarls of threads.

Immunity, therefore, is relative and depends on several intere ing details. It is not unlikely that phyletic as well as individu acclimatization plays a rôle; more important, however, are t reactions of cœlenterates to eolids, and most important the ner tocysts themselves. When touched and particularly when about to be cropped, the tentacles of a hydroid or of an actinian contract, and consequently fewer nematocysts are likely to discharge than if the tentacle remained expanded. More to the point even than this is the fact that when tentacles are cut off quickly, no discharges, or only few occur. It follows, then, that eolids are not under a heavy fire to begin with.

But such fire as they are under does not of necessity reach them, for, aside from the protection offered by the mucus which they secrete, the bodies of eolids are not the sort to be easily punctured by nettling threads. Certain experiments performed while studying the physiology of nematocysts ('09) show that a soft surface is apt to ward off the filament, and further that penetration is most easily effected when the thread is under its maximum speed at the beginning of the explosion. In order that a surface may be broken in upon, it must be very close to the mouth of the discharging nettle. Furthermore, Toppe ('09) has shown by extremely careful and interesting observations that the most common type of hydroid nematocyst is especially adapted for puncturing hard, chitinous surfaces and not soft indentible ones.

That discharge of the nematocysts after ingestion does not take place, my own observations have shown to be untrue, nevertheless such explosions are rare. In the paper on the physiology of nematocysts ('09) it was shown that the discharge of the filament is really an explosion traceable to a rise in the internal pressure of the capsule. As this rise results normally from osmosis and as the fluids in the digestive tract of eolids are probably more concentrated than those inside the nematocysts, one can easily understand why explosions within the cerata should be rare enough to have hitherto escaped detection.

Finally, it is not unlikely that immunity in the narrower sense renders ineffective such penetrations as may occur. The evidence of a "Reizgift" postulated to explain the irritations set up by nematocysts, does not appear to me as yet quite conclusive ('09). On the other hand, the evidence that there is present in nettles another poison, the hypnotoxin, responsible for the paralysis of punctured animals, seems well grounded. It is quite possible that there exists in eolids an anti-body capable of neutralizing hypnotoxin, provided this substance is not introduced in too large quantities, or in a variety to which the anti-body is not adapted. If this sort of immunity exists, then the total "immunity" of an eolid to a particular type of nematocyst is due in part to the various factors which prevent the mollusc from being punctured; in part to acclimatization, phyletic or otherwise, to the mechanical effects of such punctures as are made; and finally to the neutralization of the small quantities of hypnotoxin introduced.

With the demonstration that immunity exists, but is relative and dependent on several distinct factors, the way is paved for a consideration of the origin of the nematocyst-storing habit.

The indigestibility of the nettles renders their elimination necessary. One way in which this is accomplished is by voidance with the fæces; another by voidance through the cnidopores. Why this second method should have developed is difficult to determine. According to Hecht ('96), Calma glaucoides is a typical eolid except in two respects; it has neither cnidophores nor nematocysts. Calma cavolinii, the only other member of the genus has cnidophores filled with nettles. As C. glaucoides in all probability did not branch off from the common eolid stock before the introduction of nettles, and as its habit of feeding upon the eggs and embryos of shore fish, such as Cotta, is, with equal probability secondary, it seems likely that the abandonment of the nematocyst habit is quickly followed by degeneration of the accessory organs of elimination. If this be true, one may infer that the presence of nematocysts influences the production of cnidophores, much as an insect sting may bring on, in a plant, the formation of galls.

I do not know what it is in nematocysts that renders their prompt removal from the digestive tract proper, desirable, nor whether the immediate end, whatever it be, is accomplished by the means employed. That elimination, or at least storage, is somehow useful, while not absolutely proved, is certainly probable, for the idea that it is profitless or harmful is difficult to harmonize with the complexity of the structures and processes involved. It must be borne in mind that a ciliated canal makes possible the easy passage of nematocysts from the digestive tract to the cnidophores; that specially adapted cnidophages store the nettles, and become converted, with the aid of interstitial cells, into cnidocysts; that these step out of line and are forced by means of a special musculature out of the cnidopore; and that there is a zone of "embryonic" tissue which furnishes a supply of cells for all the purposes of the cnidophore. In addition to elimination there are other ways in which the overloading of the cerata is prevented, for new appendages are constantly forming, and by autotomy, the largest and presumably best stocked cnidophores are frequently cast upon the slightest stimulation. Even in the absence of assignable specific reasons for the voidance of nettles it seems reasonable, in face of these facts to assume that the processes now known to occur are useful, and that elimination of nematocysts is the primary function of the cnidophore proper.

Grosvenor has given a possible history of this function and of the system of organs that carry it on. "In molluscs," he writes, "other than the cladohepatic Nudibranchs, the food is digested in the stomach, where absorption takes place . . . In Tritonia, therefore, the anus suffices for the passage of nematocysts out of the body. But in the Cladohepatica, part of the food is digested in the gastric gland, quite fresh pieces of hydroid being found in the ducts and ceratal diverticula of a recently fed Æolid. . . How Dotonids which habitually feed on hydroids and have no aperatures in their cerata, get rid of the nematocysts, I cannot say; perhaps by throwing off their cerata, which as is well known they do with great ease. When an aperture for the extrusion of nematocysts had once been acquired, it would be obviously advantageous that the distal end of the "hepatic diverticulum" should be modified to form a cnidosac where the nematocysts might be stored." With this attempt at phylogenetic explanation, and the reasons cited to support it, I agree with one exception: to me it seems more probable that the "cnidosacs" as well as the habit of storing nettles are both older than the cnidopore from which extrusion takes place, and furthermore, that primitively, eolids probably cast off their excess nettles by autotomizing the cerata.

As a mechanism for voiding nematocysts, the cnidophoral system is clearly fit. This however, does not tell us why elimination should take place, nor whether either this or the complicated machinery to secure it are more than compromise responses to the biological problem, whatever it be, that lies back of both. As long as the nematocysts were considered part of the organic make-up of eolids, a sufficient reason for their presence and behavior was found in their supposed utility; nor is the case necessarily altered by the demonstration that the nettles are visitors rather than natives in the bodies of these molluscs. There are many details also which seem to add force to the argument from utility. It is a fact that the nematocysts of eolids explode on coming into contact with sea water or a medium more dilute. It is natural to infer that they are neither more nor less effective when extruded from a Montagua, than they would have been had they exploded in the mother tissues of the Tubularia that made them. It is likewise true that the explosion of these borrowed nettles is accompanied by sensations of pain if allowed to take place on a sensitive part of the body, between the fingers or on the tip of the tongue. Moreover eolids behave exactly as though a high degree of utility attached to the nettling organs. When stimulated, thermally or mechanically the nematocysts are extruded, and explode in great numbers. Grosvenor remarks:

> "No one can have witnessed the reaction of an Aeolid to various stimuli . . . without being convinced that the cerata are used as a means of defence. The body is contracted, the head being often nearly telescoped into the trunk while the cerata are erected and waved about, especially in the direction of the foreign body, and are often considerably extended.

Under these circumstances Grosvenor nevertheless did not witness the actual extrusion of nematocysts. Cuénot ('07), in discussing the same subject, writes:

> "Lorsqu'on touche un Eolidien avec une baguette de verre, il prend aussitôt une attitude particulière; les papilles s'érigent, s'allongent et se tournent autant que possible vers le point lésé, comme si elles s'orientaient pour cribler l'ennemi de nématocystes; mais en réalité, comme le dit justement Grosvenor, il y a très peu ou même pas du tout

nématocystes rejetés au dehors par la contraction des sacs. Cette attitude est donc purement émotive, et n'a pas d'effet défensif direct. Ce n'est que lorsqu'on tracasse violemment l'animal, ou mieux encore lorsque les papilles sont arrachées et comprimées, qu'il sort des sacs une masse de cellules nématophages et de nématocystes, qui explosent aussitôt. Il y a de très bonnes raisons, tirées du mode de fonctionnement des nématocystes, pour croire que cette explosion non dirigée ne peut avoir qu'un effet insignifiant . . . ; mais laissons cela et essayons des expériences."

In combats with its own kind it is not surprising that the dorsal appendages should be attacked, for not only are eolids "immune" to the nematocysts of the cerata, but these on stimulation are erected and project like the quills of a porcupine. The enemy comes unavoidably into contact with them. It is surprising, however, to witness exactly the same thing when a blennie meets an eolid. If the blennie is hungry it behaves in a seemingly pugnacious manner, darts at the eolid, seizes a mouth full of cerata, and pulls and twists them off as though they were tid-bits. As the blennie is one of the commonest denizens of regions inhabited by eolids we have here one instance at least, in which the defensive value of the nematocysts can be discounted.

Many experiments with Fundulus heteroclitus were made. In general this fish, in the first trial of an experiment will take an eolid, but almost instantly regurgitates the captive. Contrary to my earlier statement ('06), a second or even third trial may be made, depending on the degree of hunger, and if the Fundulus is very hungry, the eolid may finally be swallowed permanently. As the cerata are cast during these successive captures and regurgitations, it might be that the final retention is due to the loss of the nematocysts which at first rendered the eolid disagreeable.

Further experimentation shows that this view is untenable, for if eolids completely devoid of cerata, or forms naturally without nettles are used, a fundulus will repeat all the performances which it goes through when dealing with a normal individual bearing nematocysts. Leaving aside altogether the nettleless nudibranchs, the eolids employed in these experiments were not so seriously crushed during the trials that cost them their cerata, that the behavior of the fish could be due to nematocysts in other parts of the animal, so that obviously unpalatability to certain fish is a common property of nudibranchs, and by no means limited to the nettle-bearing species or individuals.

If these results, which are in complete harmony with those of Cuénot, are surprising, this is largely due to a common misapprehension as to the nature and significance of nematocysts. Whenever the word nematocyst is employed, it not unnaturally calls to mind the stinging artillery of a Discomedusa, or of a Portuguese man-of-war. Such armament is quite misleading, for its tremendous effects are by no means typical. Indeed the forms from which nudibranchs derive their nematocysts are themselves subject to attacks from the same fish which under certain circumstances will devour an eolid, and if the nettles of Eudendrium and of Tubularia do not protect them against browsing blennies, pinfish, and minnows, it is not to be expected that the same nettles transferred to another animal will gain in effectiveness.

In addition, the observations of Toppe ('09) suggest an important clew. Apparently the structural specialization among nematocysts is accompanied by specialization of function; for while some kinds serve as nettles, others are prehensile organs, and have nothing whatever to do with the infliction of wounds. These facts are very significant, for, aside from correcting a misconception widely spread, as to nematocysts in general, we now know definitely how the various kinds function and how beautifully each is adapted to certain specific ends. The adaptiveness of these structures is not in themselves, but comes about as the result of the habits of the animals producing them. It would be surprising indeed if a nematocyst, adapted to curl round the chitinous hairs of a copepod should be protective when exploding in the mouth of Fundulus.

Toppe's results suggest further that defense is not the original function of nematocysts, but that they are primarily organs of prehension for the purpose of entangling prey. From this standpoint it appears that the nettling function proper is secondary, and that in certain highly specialized Cnidaria, nematocysts subserving the secondary function have replaced the more primitive prehensile organs. As eolids feed on cœlenterates provided with large numbers of prehensile nematocysts, the observed inadequacy of the protection afforded is neither more nor less than one might expect.

On the basis of experiments similar to those I have presented in brief, Cuénot has concluded that the defensive value of eolidian nematocysts is slight. This inference, which the behavior of various fish makes valid receives additional support from the observations of Toppe and the conclusions to be drawn from them. It would be a mistake however to postulate complete inadequacy, for unquestionably the presence of nematocysts of all kinds in the cnidophores gives the cerata certain gustatorial peculiarities, which otherwise they would lack. When crushed on the tip of the tongue the sensation produced by a ceras is not unlike that of Tabasco Sauce. If this is added to the peculiarity, whatever it be, that renders nudibranchs in general distasteful to fish, we may conclude that a slight degree of defensive value attaches to the borrowed nettles.

According to Garstang's view ('88), shared by Grosvenor, the bright colors of the cerata serve "to direct the experimental attacks of young and inexperienced enemies to the non-vital papillæ, and away from the vital and inconspicuously colored parts of the body." The habit of responding to stimulation by erection, insures that the cerata shall bear the brunt of any attack whatever the experience of the enemy. As autotomy takes place with the greatest ease, it often happens during an encounter, that an eolid is obscured by a cloud of unattached brightly colored appendages, which, since their owner resembles the background, are certain to catch the eye of the enemy. Granted that the cerata are at least as distasteful as the body of the eolid; that in addition the presence of certain nematocysts adds even slightly to the disagreeable qualities, and one has in hand perhaps one of the minor factors in the persistance of the nematocyst-habit.

SUMMARY

The nematocysts of eolids are derived from cœlenterates, and are not to be looked upon as an instance of homoplastic evolution. Their defensive value is slight, partly because nudibranchs are distasteful to their enemies even in the absence of nettles, and partly because many of the ingested nematocysts are adapted to meet exigencies in the lives of cœlenterates which do not arise in those of eolids. A reason other than utility must therefore be discovered in order to explain the origin of the nematocyst-storing habit.

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PAMPHLET COLLECTION

NOTE ON THE DEVELOPMENT OF AMPHIBIAN LARVÆ IN SEA-WATER

Charles

OTTO GLASER

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NOTE ON THE DEVELOPMENT OF AMPHIBIAN LARVÆ IN SEA-WATER

HAT the amphibia are poisoned by common and hence geographically restricted to res free from this substance, is a general beapparently so well supported by observaand experiment, that the contradictory ence brought forward recently by Pearse[1] s at first sight enigmatical. However his very of frog larvæ in three pools of an o, or small creek opening into Manila Bay, pable of explanation although, because of tic difficulties, it is impossible to carry out necessary experiments quantitatively on s which do not live equally well in fresh salt water.

After	32	21	24	24	24	hours
in NaCl	.053125	.10625	.2125	.425	.85	per cent.
there were	15	14	12	10	0	survivors.

Time of acclimatization 101 hours.
Average strength of preparatory solution .199 per cent.

e three analyses reported by Pearse are trictly comparable, and in the calculation e NaCl from the total Cl no standard of ence is given, nevertheless it follows from culation on the basis of Forchheimer, that solutions dealt with must have been, as d by Pearse, roughly, 2.6 per cent., 2.1 cent. and 1.1 per cent. NaCl respectively. the larvæ were found in a tidal area e fresh and salt water meet, it is not probthat they were exposed continuously durheir development to the amounts of NaCl , but it is probable that they developed in a medium never free from this salt, and that despite the fact that .6 per cent. is ordinarily sufficient to prevent gastrulation.

Acclimatization to Osmotic Pressure.—A dilution of .85 per cent. NaCl (.25 per cent. less than the weakest solution reported) causes the death of 87 per cent. of the larvæ of *Rana pipiens* exposed to it for eighteen hours, whereas a solution of twice this strength kills 97 per cent. in three hours, and is therefore six times more fatal than the weaker solution. This fatality, however, depends on osmotic pressure, for both solutions brought about shrinkage, and an associated slowness and feebleness of reaction to touch. In the stronger solution these symptoms were more pronounced, and came on more quickly than in the weaker. Death resulted, therefore, probably more from dehydration than from the specifically poisonous effects of the NaCl.

These results led to an attempt at acclimatization by successively halving the .85 per cent. solution, until only one sixteenth its original strength. With this three series of tests were begun, involving fifteen larvæ. The results are given in tabular form above.

From control observations I found that 24 hours is just about the limit of endurance in the .85 per cent. solution, whereas without acclimatization 18 hours is the limit. Life, therefore, can be prolonged one third by a preliminary slow passage through weaker solutions. From this I conclude that the ability of amphibian larvæ to exist in sea water depends on their not being introduced into the stronger solutions too suddenly. This desideratum can certainly be fulfilled in an estero. These experiments leave entirely open the question of racial acclimatization which is probably of great importance.

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[1] earse, A. S., "Concerning the Development of Tadpoles in Sea Water," *Philippine Journal ience*, Vol. VI., No. 4, Section D.

The Antagonistic Effects of Calcium.—As Loeb[2] has repeatedly pointed out, very small quantities of Ca suffice to antagonize the poisonous effects of NaCl. Thus he found that in 100 c.c. of 5/8 M NaCl the eggs of *Fundulus* failed to develop, whereas when he added 4 c.c. of an M/64 $CaSO_4$ solution, 75 per cent. of the eggs formed embryos. From this it follows that one molecule of $CaSO_4$ is sufficient to antagonize 1,000 molecules of NaCl. It does not of course necessarily follow that this will prove true with other organisms, nor must we forget that other ions have been shown to antagonize the Na, but the antagonism of the Ca against the Na is so strong in the case of *Fundulus* that it is only natural to try this first in the case of the frog larva.

The deleterious effect of a 1.7 per cent. NaCl solution to which .09 per cent. $CaSO_4$ had been added proved, as one might expect, greater than that of the pure NaCl solution. Even when diluted one half 93 per cent. of the larvæ died in 13 hours. However, preliminary treatment with more dilute solutions, even for a relatively short time, not only practically doubled the time during which the larvæ can endure the .85 per cent. solution, but only 7 per cent. died in 24 hours. (See table, p. 3.)

After	12	11	11	24	hours
in NaCl	.10625	.2225	.425	.85	per cent.
in $CaSO_4$	.005625	.01125	.0225	.045	per cent.
there were	15	15	15	14	survivors.

Time of acclimatization 34 hours.
Average strength of preparatory solution in Na .248 per cent.
Average strength of preparatory solution in Ca .013 per cent.

Comparing this result with the first acclimatization, it is seen that when Na and C are present roughly in the proportion of 50 t 1, the tadpoles may be acclimated to th .85 per cent. solution about three times a fast as in the absence of the Ca, and furthe more that 93 per cent. of the larvæ so treate can endure this solution for 24 hours, wherea in the absence of Ca, none survive. Al though the concentrations here dealt with ar lower than those reported by Pearse, althoug not much lower than his weakest solution, th proportion of Na to Ca is, according to Forch heimer, identical.

Other Antagonisms.—In a series of investi gations Loeb and Wasteneys[3] have demon strated and measured the antagonistic effect of KCl and NaCl on *Fundulus,* so that in view of the fact that the findings with reference t Ca can be verified on the frog larva, it seem reasonable to assume similar relations for th K and Na. For Mg this is certainly true, al though its antagonism to Na is much les marked, as the results given below show.

Comparison with the results with pur NaCl shows that the presence of the Mg en ables the larva to withstand a solution of in creasing concentration with average strengt of .199 per cent. NaCl for 104 hours withou a single death, whereas in the absence of Mg 33 per cent. die in 101 hours. Both in th presence and absence of Mg, however, ther were no survivors in the .85 per cent. solutio after 24 hours, which shows that the Na–M antagonism is less pronounced than that b tween Na and Ca.

While these experiments give some insigh into the conditions of survival for frog larv in sea water, the actual circumstances a probably not as simple as one might at fir conclude, for granted an antagonistic actio

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Dynamics of Living Matter,” p. 47.

[3] *Biochemische Zeitschrift,* Bd. 31 and 32.

After	24	24	35	21	24	hours
in NaCl	.053125	.10625	.2125	.425	.85	per cent.
in $MgCl_2$	.0053125	.010625	.02125	.0425	.085	per cent.
there were	15	15	15	15	0	survivors.

Time of acclimatization 104 hours.
Average strength of preparatory solution in Na .199 per cent.
Average strength of preparatory solution in Mg .0199 per cent.

tween Na and K, Na and Ca, and Na and
g, it does not follow that in a solution in
ich all these salts are present, the total
tagonistic effect toward Na could be repre-
ited by the formula, Na *vs.* (K + Ca + Mg)
ions capable of antagonizing the Na may
tagonize each other. The existence of these
ccessory" antagonisms has been demon-
ated by Loeb and Wasteneys for *Fundulus*.

After	21	21	25	24	24	hours
in NaCl	.053125	.10625	.2125	.425	.85	per cent.
in $CaSO_4$	.0028125	.005625	.01125	.0225	.045	per cent.
in $MgCl_2$	.0053125	.010625	.02125	.0425	.085	per cent.
there were	15	15	15	15	12	survivors.

Time of acclimatization 120 hours.
Average strength of preparatory solution in Na .199 per cent.
Average strength of preparatory solution in Ca .0105 per cent.
Average strength of preparatory solution in Mg .0199 per cent.

the case of the frog embryos I found an
agonistic action between Mg and Ca, for
ough both these ions antagonize the Na,
a solution containing all three is a less
orable one for the embryos than one con-
ing only Na and Ca. The experiments
which this statement is based are tabulated
ve and can be compared with the earlier
s.

'onclusion.—The ability of amphibian eggs
levelop in sea water is dependent on the principle of ionic antagonism. In addition to this, however, their power of acclimatiza- plays an important rôle, for it not only enables them to withstand the passage from dilute to strong solutions, but the opposite process as well. Thus larvæ which have just reached a point where they fail to react to tactile stimuli in solutions which do not bring about dehydration, either because the solutions are too weak, or because the larvæ have been acclimated, will if transferred to fresh or distilled water recover in from one to two hours. If in addition to this we remember that the species found by Pearse is probably racially acclimated to the conditions under which it lives, his findings do not appear inexplicable.

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REFLECTIONS ON THE AUTONOMY OF BIOLOGICAL SCIENCE

PROFESSOR OTTO GLASER

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REFLECTIONS ON THE AUTONOMY OF BIOLOGICAL SCIENCE

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Introductory

If the knowledge of facts and comprehension of principles by certain writers had been adequate, and others had freed their minds from the survivals of animism, the taxonomic position of biology in the scheme of knowledge would appear uncertain to no one. Prolonged and extensive inkshed however have surrounded this question with much unnecessary difficulty and confusion. Some claim that biology can not properly find a place among the sciences at all; others, that if our science is nothing more than physics and chemistry, it can have no right to independent existence; and finally, the vitalists postulate an absolute autonomy based on a specific principle.

Biological Prediction

Merz, Enriques,[1] and other present-day writers on the systematics of biology dwell at length on the fact that within the realm of the living, very strange and unexpected events take place. From the protozoans, human beings can hardly be inferred; the chromosomal complex, on account of the variations and surprising similarities of its constituent elements, fails to tell us whether we are dealing with sister species or with forms as remote as snails, frogs, ferns and mice. Because one crustacean is positively heliotropic, it does not follow that the next one, even if the species be identical, will respond in like manner, nor because one child in a family has blue eyes can we conclude that its parents or brothers and sisters have eyes of the same color. A dog or a man may be friendly to-day and vicious to-morrow under similar external circumstances. Irregularities such as these, our informants tell us, should quench the ardor of the dullest, and to convince us still further of the inadequacy of our materials for science they point to rational mechanics, a domain free from ambush, and pervaded by an order in which only the foreseen and predictable find a place.

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[1] Merz, John Theodore, "A History of European Thought in the Nineteenth Century"; Enriques, Federigo, "Probleme der Wissenschaft."

The juxtaposition of these two disciplines is not only unparliamentary, but unfortunate. Inasmuch as rational mechanics deals with abstractions and has only the slightest objective basis, it can have no materials comparable with the contents of any natural science. On the contrary, it is a method of thinking. Thinking is a phenomenon of consciousness; and consciousness, a biological event. If, therefore, the mechanic produces an orderly and coherent system in which one thing follows with certainty from another, this shows nothing else than that certain biological events, to wit, mental processes, are among the most reliable phenomena in nature.

The biologist readily concedes that he is not as weatherwise as the rational mechanic, but he does not concede that this is due either to the fundamental disorderliness of his section of nature, or because his colleague's oracular powers differ in origin from his own where he happens to possess them. As a whole man can not as yet be inferred from the protozoa, yet from the study of oxidation, secretion and digestion in unicellular organisms we could readily foresee the existence of these processes in higher forms. The conditions of the heliotropic response are such that an organism must be neither neutral nor alkaline to react positively, and one at variance with expectation can be made to do the expected by acidulation. Although half the children of brown-eyed parents may have blue eyes, this, instead of being a symptom of disorder, is in strict conformity with a law which enables us to say that two grandparents, one maternal, the other paternal, had this eye-color. The same law makes it possible to predict the proportion and sex of color-blind persons in a family in which this defect is present. The change from friendliness to viciousness in dogs and men has been traced to definite chemical and structural changes so often that it could undoubtedly be foreseen if these were known. The embryologist foretells the hour of ovulation, the obstetrician the birth of a child, the entomologist the reappearance of a brood of locusts, the ornithologist of a flock of birds, and the ichthyologist of a school of fish, with the same reasonable certainty with which the celestial mechanic predicts the return of a comet. By the behavior of *Convoluta roscoffensis,* even though far from its native haunts, it is possible to tell the state of the tides. It should never be forgotten that Weismann predicted the phenomena of maturation in the germ cells.

Because the chromosomes have at present no taxonomic importance, Merz concludes that they never can have, and that biological events are therefore disorderly. It so happens that the particular facts which Merz would like to predict from these bodies are not related to what we know about them in a manner so intimate that in the present state of science prediction here would be any more reasonable than in the absence of wind to judge the weather from a bonfire. There are no reasons to doubt that if we knew accurately the chemical structure of the chromosomes, instead of merely their general composition, number, size and shape, we could tell the species, and perhaps predict their composition in related species, much as the organic chemist predicts the make-up of one compound from another. Even now, the physiological state of the cell, and in numerous instances its kind, as well as the sex of the individual from which it was taken, can be determined from the chromosomal complex.

How the rational mechanic acquired his prophetic powers can be answered by considering the development of geometry. Are we expected to believe that from the capable of showing that this animal is a cow. Even if we reduce its tissues to their constituent chemical elements, and, not content with this, continue until we have shown that a horse is entirely composed of electrons, and their activities, how could this show that a horse is not a horse? If therefore resolution can detract nothing from the things analyzed, it is clear that if these are in any way unique, they will be no less so after this process than before. The only question which can be at issue is whether living things are, or are not, unique. To this only an affirmative answer is possible.

To reason with defectives is unprofitable for they have no organ with which to perceive the qualities by which we differentiate between the organic and the inorganic. If we ask ourselves how we make this distinction we naturally think of the fact that living things are machines with the power, as Loeb puts it, of automatic self-preservation and reproduction. All the wonderful processes for which in the aggregate this simple formula stands divide animals and plants sharply from matter not alive and constitute the specific basis for the autonomy of our science. This autonomy is nothing metaphysical, or absolute, but practical, like the autonomy of physics, chemistry, astronomy and geology.

HISTORICAL BACKGROUND OF THE POSTULATED ABSOLUTE AUTONOMY

In their analyses of living things, modern biologists make use of only one practical method, but they apply it from two distinct points of view, and since the significance of phenomena in general depends on the point of view, the whole meaning of the science hangs in the balance. The validity of these theoretical standpoints, therefore, should be tested as carefully as the proposed site of an observatory.

Unfortunately the issues at stake can not be properly apprehended without some knowledge of their history. To begin with Aristotle, and the few Ionians and Eleatics who preceded him, however, does not give us the needed historical background, for the impression that Aristotle was a primitive man, or that science was born in Greece, is surely wrong. Scientific knowledge began with the human race.

Although the thoughts of early men are for the most part unrecorded, study of the primitive men living to-day shows conclusively that the problem of the origin and nature of life is realized by the savage. In the lore of medicine men, magicians and seers, scientific knowledge, theories and beliefs, fuse into an alloy which, despite the varied conditions of its genesis and growth, presents remarkable homogeneity. In this cultural amalgam the attempt is made to explain the difference between a dead man and a live one, by means of "a thin unsubstantial human image, in its nature a sort of vapor, film or shadow; the cause of life and thought in the individual it animates; independently possessing the personal consciousness and volition of its corporeal owner, past or present; capable of leaving the body far behind to flash swiftly from place to place; mostly impalpable and invisible, yet also manifesting physical power, and especially appearing to men waking or asleep as a phantom separate from the body of which it bears the likeness; continuing to exist and appear to men after the death of that body; able to enter into, possess and act in the bodies of other men, of animals and even of things."[2]

These conclusions, drawn from the experience of dreaming, are not much more primitive than the opinions prevalent during the middle ages and surviving in the shadows of church spires to-day. Now and again, however, revolutionary teachings arose, and the most significant of these for our immediate purposes are the doctrines of René Descartes.

In his splendid history of biological theories, Rádl[3] has traced with considerable detail the fortunes of the controversy set going in 1644 by the "Principes de la Philosophie" at a time when practically all men were vitalists. During the seventeenth and eighteenth centuries this contest engaged the ablest minds, yet mechanism achieved no decisive victory, but only an increase in the number of its followers, and the substitution of the original soul in vitalism by the life force of Müller, itself destined to elimination in the nineteenth century by supersession, largely by neglect, and by direct experiments on vital energetics.

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[2] Tylor, Edward B., "Primitive Culture."

[3] Rádl, Emil, "Geschichte der Biologischen Theorien."

Émil du Bois Reymond stands out as the champion of mechanism during this period, although the limitations of his materialism led him to classify the problem of life with six other insoluble riddles. Lotze overthrew the life force with arguments, substituted a purposeful preformation in the germ, and protected it from further harm by asserting that to inquire into its origin was unscientific. Fechner and Preyer attempted to clear the atmosphere by insisting that life is fundamental and the real problem the origin of the inorganic. Virchow contributed the idea of a mechanism superimposed upon that already known, and this in the hands of his successor Rindfleisch became a theory of atomic consciousness. In the seventies, however, ghostly voices fell upon deaf ears, for under the leadership of Darwin a seemingly satisfactory natural explanation of adaptation forced the mechanistic pendulum to its highest point.

While this period of scientific development proved fatal to naturalistic vitalism, metaphysical not only survived, but during the latter-day Darwinian decadence and reconstruction has again emerged, leaving behind some of the crudities of its forerunners, and apparently purged of ghosts. A change of names, however, does not constitute a change of nature. The ghosts, more rarefied than ever, are with us still, only to-day we call them Entelechies, Dominants, Psychoids and Élan Vital.

ANALYSIS OF NEO-VITALISM

Plate[4] finds in neo-vitalism four fundamental postulates about which discussion must necessarily center. These propositions are as follows:

I. Neither now nor in the future can the organism be explained by chemistry and physics without a remainder.

II. There is an absolute distinction between dead and living matter; in the inorganic world the law of causation holds, but in the organic causation holds together with a unique law.

III. The uniqueness expresses itself in this, that every organic process is final (teleological), that is, governed by immanent purposefulness.

IV. The cause of this finality, in so far as the vitalists are not agnostic, is (*a*) a psychical factor; (*b*) a metaphysical factor.

POSTULATE I

Neither now nor in the future can the organism be explained by chemistry and physics without a remainder.

Nothing could be more physical and chemical than the analysis of the whole universe into a system of electrons. When such resolution has been accomplished and every known chemical element has been shown to be a special case of corpuscular movement, the organic world and all that characterizes it will be expressible in terms of electrons if this mode of expression should appear serviceable. Would it not remain true, however, that hydrogen is hydrogen, and oxygen, oxygen? Even if these gases were proved to be configurations of essentially similar corpuscles, they would nevertheless continue to be individually different, and those so inclined would find it possible to found separate sciences of hydrogenology and of oxygenology, and these subjects would be autonomous. Does any one conclude from this that the mechanist is not fit to deal with these matters? Or that his methods are fundamentally inadequate? Yet the argument of those who would cast mechanism out of biology is identical. Resolution leaves intact uniqueness wherever found, and the declaration that this is true of the organism is a platitude.

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[4] Plate, Ludwig, "Darwinsches Selektionsprincip," 3d ed.

POSTULATE II

There is an absolute distinction between dead and living matter; in the inorganic world, the law of causation holds, but in the organic, causation holds together with a unique law.

The second part of this proposition will be considered in connection with postulate III. To the first part the mechanist subscribes heartily, but adds that in his experience the distinction between hydrogen and oxygen is equally absolute.

POSTULATE III

The uniqueness expresses itself in this, that every organic process is final (*teleological*); *that is, governed by immanent purposefulness.*

In discussing postulate III, all that is needed is (*a*) to sound its logical consequences; (*b*) to inquire how it agrees with observations on individual and racial finality; and lastly, (*c*) to expose the psychology of the teleological idea itself.

(*a*) From the harmony between the organic and the inorganic, Driesch concludes that "nature is nature for a purpose." If the whole universe, however, is governed by immanent purposefulness what becomes of the distinction between the organic and the inorganic? In a purposive system the teleological nature of any particular event or group of events can not be inferred, for purposefulness can only be recognized by comparison with purposelessness. Thus general teleology denies the existence of half the materials for the inference of the very thing on which it bases itself, and with the best intentions in the world, and without in any way seeming to sense it, vitalists themselves have not only disarmed teleology in the realm of the living, but have made the principle scientifically impossible.

(*b*) Were every organic event final or purposeful, functional adjustment, training and education would be unnecessary and impossible. Jennings[5] tells us:

> How the relations that impress us as teleological were brought about, constitutes undoubtedly a set of most difficult problems. But to keep us from despairing, we find this process taking place in the lives of individuals in a manner that can readily be studied. This is in the formation of habits. In the formation of habits, we see that the organism at first does not react in a way that impresses us as teleological, while later it does, and we can watch the process change from one condition to the other, and discover how it is causally determined. Since then a method of action that appears to us teleological is produced in an intelligible way under our very eyes, in the lifetime of the individual, there is no reason why we may not expect to find out how teleological relations have been brought about in the life of the race when we have actually made a start in the study of the physiology of racial processes. It seems clear that the apparent relation of a present process or structure to something that comes later in time is always due to the fact that this future something has in fact acted upon the organism in the past. The present condition fits the future condition only because of a certain constancy in the universe, through which the "something past" reappears again in the future.

The ability to make functional adjustments of this character is only a special case of automatic self-preservation, and is found in all organisms because those devoid of it are for this very reason eliminated and consequently remain largely unknown. Paleontology is the science that deals chiefly with these failures. How many organisms have been unable to make the necessary adjustments is attested by the great number of extinct animals and plants; how many are failing to-day is shown by every rapidly vanishing species, as well as by many experiments and special observations. Several of the mutants of de Vries have for one reason or another proved indurable, whereas Loeb[6] has pointed out that faulty organisms must frequently arise, although we only become aware of them under exceptional conditions.

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[5] Jennings, Herbert S., "Diverse Ideals and Divergent Conclusions in the Study of Behavior in Lower Organisms," *American Journal Psychology*, Vol. XXI.

[6] Loeb, Jacques, "The Mechanistic Conception of Life," *Pop. Sci. Mo.*, Vol. LXXX.

Moenkhaus found ten years ago that it is possible to fertilize the egg of each marine bony fish with sperm of practically any other marine bony fish. His embryos apparently lived only a very short time. This year I succeeded in keeping such hybrid embryos between distantly related bony fish alive for over a month. It is therefore clear that it is possible to cross practically any marine teleost with any other.

The number of teleosts at present in existence is about 10,000. If we accomplish all possible hybridization 100,000,000 different crosses will result. Of these teleosts only a very small proportion, namely, about one one-hundredth of one per cent., can live. It turned out in my experiments that the heterogeneous hybrids between bony fishes formed eyes, brains, ears, fins and pulsating hearts, blood and blood vessels, but could live only a limited time because no blood circulation was established at all—in spite of the fact that the heart beat for weeks—or that the circulation, if it was established at all, did not last long.

The possibility of hybridization goes much further than we have thus far assumed. We can cause the eggs of echinoderms to develop with the sperm of very distant forms, even mollusks and worms (Kupelwieser): but such hybridizations never lead to the formation of durable organisms.

It is therefore no exaggeration to state that the number of species existing to-day is only an infinitely small fraction of those which can and possibly occasionally do originate, but which escape our notice because they can not live and reproduce. Only that limited fraction of species can exist which possesses no coarse disharmonies in its automatic mechanism of preservation and reproduction. Disharmonies and faulty attempts in nature are the rule, the harmonically developed systems the rare exception. But since we only perceive the latter we gain the erroneous impression that the "adaptation of the parts to the plan of the whole" is a general and specific characteristic of animate nature, whereby the latter differs from inanimate nature.

If the structure and the mechanism of the atoms were known to us we should probably also get an insight into a world of wonderful harmonies and apparent adaptations of the parts to the whole. But in this case we should quickly understand that the chemical elements are only the few durable systems among a large number of possible but not durable combinations.

(*c*) Overlooking for the moment the obvious difficulties of the assumption, we can be certain that the idea of teleology would never have entered the biologist's head were he not himself a living thing. Since this is the case, however, his interest in life exceeds all others, and he attends to the processes that make life possible only because of their resultant. Inasmuch as the latter occupies the focus of his mind, he wrongfully reasons backward from results to processes, and finding in these none that might have rendered the cherished product impossible, concludes that the processes were all along aiming at what, from his standpoint, is the end. Clearly the conclusion has only an anthropocentric basis.

POSTULATE IV

The cause of this finality, in so far as the vitalists are not agnostic, is (a) a psychical factor; (b) a metaphysical factor.

Since biological finality is an anthropomorphism, a discussion of the supposed teleological factors is futile. Inasmuch, however, as psycho-vitalism has its counterpart in psycho-mechanism, the fallacy common to both may be pointed out.

(*a*) To reflect mind into the cell, and so reflected to use it as an explanation of what the cell does, is the method of primitive animism. Quite apart from the fact that the existence of mind, so far, at least, has been demonstrated only in the case of certain higher animals, but not at all for the lower, or the developmental stages of the higher, as an explanation it can have no title to serious consideration since it is itself one of the elements of the automatic self-preservation which it is the aim of biology to analyze. To interpret something we do not understand in terms of something else which at present we understand even less, may give temporary comfort to some minds, but the ideals of scientific explanation call for the reverse process.

(*b*) The difficulties of Driesch's style are such that many biologists refuse to read his books. For this reason I have made from one of them[7] a series of extracts to serve as illustrative material. The italics are not mine.

DRIESCH'S ENTELECHY

> Entelechy or the psychoid has nothing of a "psychical" nature. (P. 138.)
>
> We indeed are in a rather desperate condition with regard to the real analysis of the fundamental properties of morphogenetic, adaptive, and instinctive entelechies: for there *must* be a something in them that has an analogy, not to knowing and willing in general, as it may be supposed to exist in the primary faculties of psychoids, ***but to the willing of specific unexperienced realities,*** and to knowing the specific means of attaining them. (P. 142.)
>
> To build up the organism as a combined body of a typical style is the task of entelechy; entelechy means the faculty of achieving a "forma essentialis"; being and becoming are here united in a most remarkable manner; time enters into the Timeless, *i. e.,* into the "idea" in the sense of Plato. (P. 149.)
>
> There is first the entelechia morphogenetica, and after that the entelechia psychoidea and the latter may be discriminated as governing instincts and actions separately. Furthermore the different parts of the brain, such as the hemispheres and the cerebellum in vertebrates, may be said to possess their different kinds of entelechy.
>
> In fact we may speak of an order concerning the rank or dignity of entelechies, comparable with the order of ranks or dignities in an army or administration. But all entelechies have originated from the primordial one and in *this* respect may be said to be one altogether.
>
> Now the primordial entelechy of the egg not only creates derived entelechies, but also builds up all sorts of arrangements of a truly mechanical character; the eye, in a great part of its functioning is nothing but a camera obscura, and the skeleton obeys the laws of inorganic statics. Every part of these organic systems has been placed by entelechy where it must be placed to act well in the service of the whole, but the part itself acts like a part of a machine.
>
> So we see finally that the different forms of harmony in the origin and function of parts that are not immediately dependent on one another, are in the last resort the consequence of entelechian acts. The entelechy that created them all was harmonious in its intensive manifoldness; the extensive structures which are produced by it are ***therefore*** harmonious too. In other words there are many processes in the organism which are of the statical-teleological type, which go on teleologically or purposefully on a fixed machine-like basis, but entelechy

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[7] Driesch, Hans, "The Science and Philosophy of the Organism," Gifford Lectures, 1908.

has created this basis, and so statical teleology has its source in dynamical teleology.

We now see the full meaning of the statement that entelechy is an "intensive manifoldness" realizing itself extensively; in other words, we know what it means to say that a body in nature is a living organism; we have given a full descriptive definition of this concept. (Pp. 150–151.)

Any *single* spatial occurrence induced or modified by entelechy has its previous *single* correlate in a certain *single* feature of entelechy as far as it is an intensive manifoldness. (P. 154.)

Entelechy may be aroused to manifestation by a change in bodily nature, such as is effected by fertilization, or by some operation, or by some motor stimulus; on the other hand, entelechy may on its own part lead to changes in bodily nature. (P. 156.)

It is the essence of an entelechy to manifest itself in an extensive manifoldness: all the details of this extensive manifoldness depend upon the intensive manifoldness of the entelechy, but not upon different spatial "causes." (P. 157.)

Entelechy lacks all the characteristics of quantity; entelechy *is order* of relation and absolutely *nothing* else; all the quantities concerned in its manifestations in every case being due to means which are used by entelechy, or to conditions which can not be avoided. (P. 169.)

Entelechy, as far as we know, at least, is limited in its acting by many specificities of inorganic nature, among which are the specificities included under the phrase "chemical element." (P. 179.)

Entelechy is also *unable* to cause reactions between chemical compounds which never are known to react in the inorganic world. In short entelechy is altogether *unable* to create differences of intensity of any kind.

But entelechy *is* able, so far as we know from the facts concerned in restitution and adaptation, to *suspend* for as long a period as it wants any one of all the reactions which are *possible* with such compounds as are present, and which would happen without entelechy. (P. 180.)

Entelechy though not capable of enlarging the amount of diversity of composition of a given system, is capable of augmenting its diversity of distribution in a regulatory manner, and it does so by transforming a system of *equally* distributed *potentialities* into a system of *actualities* which are *unequally* distributed. (P. 192.)

Entelechy . . . is a factor in nature which acts teleologically. It is an intensive manifoldness and on account of its inherent diversities it is able to augment the amount of diversity in the inorganic world as far as distribution is concerned. It acts by suspending and setting free reactions based upon potential differences regulatively. There is nothing like it in inorganic nature. (P. 205.)

Entelechy is an elemental factor of *nature* conceived to explain a certain class of natural phenomena. (P. 206.)

You may say if you like that entelechy, when turning a mass particle, acts upon it at right angles to its path—this kind of action requiring no energy, but even thus there would be only a pseudo-obedience to the laws of real mechanics, since entelechy must be regarded here as non-energetical and as interfering with inertia at the same time. (P. 223.)

Entelechy is affected by the accomplishment of its own performance, in acting as well as in morphogenesis. (P. 228.)

In order that adaptation may happen, the fundamental state of the organism must be disturbed in its normality; this fact affects or calls forth entelechy. (P. 229.)

Entelechy is *affected* and thus called into activity by *changes of any normality* governed by it which are due to external causes and these changes *do not affect entelechy as a mere sum of changed singularities, but as changes of normality as a whole.* (P. 232.)

Entelechy is affected by and acts upon spatial causality as if it came out of an ultra-spatial dimension; *it does not act in space, but it acts into space.* (P. 235.)

Entelechy is an agent acting manifoldly without being itself manifold in space or extensity. Entelechy then is only an agent that arranges, but not an agent that possesses quantity. (P. 250.)

Entelechy is something different from matter and altogether opposed to the causality of matter. (P. 255.)

May not entelechy be called a " substance " in the most general philosophical sense of the word, that is, in the sense of a something irreducible, which remains the always unchangeable bearer of its changeable qualities. (P. 256.)

Entelechy has the power of preserving its specific intensive manifoldness in spite of being divided into two or more parts. (P. 257.)

Entelechy therefore can not possess a " seat." (P. 258.)

At present the question whether entelechy is a " substance " must remain as open as the previous question about the relation of entelechy to causality. . . . Entelechy was a kind of " quasi " causality, and now may be said to be an enduring " quasi-substance." (P. 260.)

Entelechies, though transcending the realm of the Imaginable, do *not by reason of their logical character as such* form constituents of metaphysics in the sense of something absolute and independent of a subject. (P. 320.)

Entelechy is *alien* not only to matter but *also to its own material purposes.* (P. 336.)

Mir wird vor alle Dem so dumm
Als ging mir ein Mühlrad im Kopf herum!

CONCLUSION

I have tried to show that biological events are orderly; that a distinct problem guarantees the autonomy of the science; that the application of physical and chemical methods has no shortcomings specifically different from those met with when applied to the inorganic, and finally that vitalism in addition to being unnecessary is absurd. The question whether the modern outburst of metaphysical biology, a movement which finds favor among philosophers and psychologists, and has no small following among zoologists and botanists, is not, despite its obvious faults, sound in motive, remains to be answered. Mechanical methods, even if applicable to vital events no less than to any others, might nevertheless possess an inherent weakness discoverable only when enlisted in biological service. The only reply possible to this question is that they are the best methods which human beings can devise, for their excellencies are grounded in our structure, their deficiencies in that of the world outside.

It has been pointed out over and over again that the explanations of science never amount to more than the enumeration of the conditions under which the events in nature take place. With ultimate explanation science does not deal, not because men of science do not want to, but because in their experience nature contains nothing ultimate. The failure, therefore, of science to give us more than it does can be held up as a fault only by those who are dissatisfied with the structure of the universe. For this feeling intellectual hygiene is the only cure.

If the limitations of scientific methods are to be found in the limitations of a limitless universe, their excellencies, as instruments for the automatic preservation of life, are to be found in ourselves, for the mechanical symbols by the aid of which natural phenomena are interpreted are the easiest for us to use. The value of these symbols depends on our power to visualize, and visualization depends on sight. Is it without significance in this connection that the eye begins in the embryo earlier than any other receptor of special sense, or that sight, except perhaps by a few poets and musicians, is acclaimed the most priceless of all our senses?

If we lived in a world of phantasms, the value of sight would largely disappear, for, as Berkeley[8] has pointed out, it is an organ of anticipatory touch upon which depends our ability to avoid harmful collisions, and to bring about desirable ones. From the very beginning of our lives we see and deal with visible objects. Is it strange then that we should attempt to express all our experience in terms of the language which by our very structure and history is the most used and hence the most efficient medium of interpretation we possess?

Modern energetics has indeed discarded solid molecules and atoms, and has replaced these by constellations of electrons, yet even if the electrons are nothing more than electrical charges, they are believed to possess mass, and to have certain properties in common with visible things. Does not the physicist still draw pictures on the wall to make clear what he means? Is not a picture a visual symbol by the aid of which we understand a less familiar one? Escape is impossible, for mechanistic symbolism is grounded in our very nature, and for this reason its employment rises to the dignity of a moral act, for it involves neither more nor less than the application of our best capabilities to the best of all purposes—the interpretation of nature.

---

[8] Berkeley, George, "An Essay Towards a New Theory of Vision."

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Die Entwicklungsarbeit im Fundulusei.

VIII. Beitrag zur Energetik der Ontogenese.

Von
Otto C. Glaser (Ann. Arbor, Michig. U. S. A.).

(Aus der königl. ung. tierphysiologischen Versuchsstation in Budapest,
Direktor: Franz Tangl.)

(Eingegangen am 2. Mai 1912.)

Der Zweck meiner Versuche war, die Entwicklungsarbeit im Ei eines Knochenfisches, des Fundulus heteroclitus, in derselben Weise zu bestimmen, wie es F. Tangl¹) für das Hühnerei und Forellenei und sein Schüler K. Farkas²) für das Ei des Seidenspinners ermittelte. Wie in den Tanglschen Versuchen wurden die Veränderungen festgestellt, die während der Entwicklung des Embryo im Substanz- und Energiegehalt (Gehalt an chemischer Energie) im Eiinhalt auftreten. Aus der Differenz zwischen Anfangs- und Endstadium läßt sich dann, unter den von Tangl angegebenen Bedingungen, die Menge der verbrauchten organischen Substanz und chemischen Energie berechnen. Die wesentlichste Bedingung ist, daß während der embryonalen Entwicklung keine, chemische Energie enthaltende Substanzen dem Ei zugeführt werden (wie das z. B. beim Säugetierei geschieht) und daß keine solchen aus dem Ei verloren gehen. Beim Vogelei, Forellen- und Seidenspinnerei waren diese Bedingungen erfüllt; dasselbe konnte ich nach den Erfahrungen am Forellenei auch für das Fundulusei mit Recht voraussetzen.

Die Eier sammelte ich im Juni 1911 im Marine Biological Laboratory in Woods Hole Mass., U. S. A. Es war nicht leicht, eine zu den Versuchen nötige Menge von Eiern zu verschaffen, so daß die Untersuchung eben wegen Mangels an Material

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¹) Arch. f. d. ges. Physiol. **93**, 327; **104**, 624; **121**, 437.

²) Arch. f. d. ges. Physiol. **98**, 490.

manche Lücken aufweist. Ein Weibchen gibt beim Abstreifen höchstens einige Hundert entwicklungsfähige Eier ab. Die Eier nahm ich in der in Fischzüchtereien üblichen Weise ab, während die Spermien entweder ebenfalls durch Abstreifen oder durch Zerzupfen der Hoden einiger Männchen gewonnen wurden. Ich habe stets künstliche Befruchtung angewendet, die am besten auf trockenem Wege, d. h. ohne Zusatz von Seewasser, gelang. Es muß aber betont werden, daß selbst unter günstigen Bedingungen die künstliche Befruchtung nicht in allen Eiern zur Embryogenese führt.

Ungefähr $^1/_4$ Stunde nach dem Zusatze der Spermiensuspension wurden die Eier mehrere Male in frischem Seewasser gewaschen und dann in zugedeckten Schälchen der weiteren Entwicklung überlassen. Täglich wurden die toten oder sich nicht entwickelnden Eier entfernt und das Wasser erneuert. Eine Probe der Eier kam unmittelbar nach der Befruchtung in den Trockenschrank, während die anderen bis zum Ausschlüpfen weiter bebrütet wurden. Leider sind verhältnismäßig wenige zum Ausschlüpfen gekommen, was durchschnittlich 384 Stunden nach der Befruchtung stattfindet. Ich trocknete nämlich auch Proben der Zwischenstadien (bzw. 54, 72, 144, 169 und 140 Stunden nach der Befruchtung), diese habe ich auch zum Teil aufgearbeitet, doch müssen diese Versuche noch wesentlich ergänzt werden, ehe sich näheres über den Stoff- und Energieumsatz der Zwischenstadien berichten läßt. Vorderhand habe ich nur Anfangs- und Endstadium der embryonalen Entwicklung verglichen.

Das Trocknen der Eier geschah bei 60° im Trockenschranke und dieses Trockenmaterial diente dann zu den Untersuchungen, die ich im Laboratorium des Herrn Prof. F. Tangl in Budapest ausführte.

Bestimmt wurde an den einzelnen Proben Trockensubstanz- und Aschegehalt. Letzterer mußte sehr sorgfältig mittels des Extraktionsverfahrens ermittelt werden, da der Kochsalzgehalt der Asche ein außerordentlich hoher war. Selbst die geringe Menge Seewasser nämlich, die an den klebrigen Eihüllen haften bleibt, erhöht den Aschengehalt ganz gewaltig, und zwar in solchem Maße, daß die kalorimetrische Verbrennung öfter mißlang.

Die kalorimetrische Verbrennung zur Bestimmung des Gehaltes an chemischer Energie habe ich mittels der kleinen kalorimetrischen Bombe des Institutes, die Zaitschek[1]) geprüft hat, ausgeführt, genau so, wie es K. Farkas mit den Seidenspinnereiern machte.

Die folgende Tabelle enthält die Ergebnisse dieser Bestimmungen:

	Trockensubstanz g	Asche g	Organ. Substanz g	Chem. Substanz Cal.
1000 Eier enthalten unmittelbar nach der Befruchtung samt Eihüllen	0,660	0,185	0,475	3273
1000 Larven unmittelbar nach dem Ausschlüpfen ohne Eihüllen	0,535	0,185	0,350	2550
in den dazu gehörigen Eihüllen	0,045	—	0,045	
also sind verschwunden . .	—	—	0,080	723

Es soll zunächst bezüglich des Aschengehaltes der Eier bemerkt werden, daß derselbe wahrscheinlich durch das unvermeidlich anhaftende Seewasser, wie schon erwähnt, außerordentlich hoch ist. Natürlich ist so aus den Zahlen nicht ersichtlich, wieviel von der Asche dem Ei selbst zukommt, was aber zu kennen deshalb wünschenswert gewesen wäre, weil aus der ev. Konstanz des Aschengehaltes darauf gefolgert werden könnte, daß während der Entwicklung von dem Eiinhalt nichts verloren ging. Immerhin ist es bemerkenswert, daß ich bei Eiern verschiedenen Alters, 36 bis 142 Stunden nach der Befruchtung, für den Aschengehalt ziemlich übereinstimmende Werte gefunden habe: 0,170 bis 0,199 g, im Mittel 0,185 g. Mit diesem Mittelwert habe ich dann auch für die ausgeschlüpften Larven den Gehalt an organischer Substanz berechnet.

Was die Eihüllen betrifft, so habe ich zur Bestimmung ihres Trockensubstanzgehaltes in einer Anzahl konservierter Eier die Eihüllen sorgfältig abpräpariert und getrocknet. Ihre Substanzmenge ist sehr gering, dementsprechend auch ihr Gehalt an chemischer Energie. Eben deshalb habe ich ihren geringen Gehalt an Asche unberücksichtigt gelassen, ebenso ihren Gehalt an chemischer Energie. Der dadurch bedingte Fehler kommt ei der Berechnung der Entwicklungsarbeit um so weniger in etracht, da es sich bei letzterer doch nur um den ersten äherungswert handeln kann.

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[1]) Arch. f. d. ges. Physiol. 121, 128, 1908.

Aus den angeführten Zahlen ergibt sich, daß während der ntwicklung von 1000 Funduluslarven von der Befruchtung s zum Ausschlüpfen der Larven

$$0,475 - 0,390 = 0,080 \text{ g organische Substanz und}$$

$$3275 - 2550 = 723 \text{ g-cal chemischer Energie}$$

braucht wurden. Die Entwicklungsarbeit von 1000 Fundulusven beträgt also 723 g-cal.

Für den Vergleich mit der Entwicklungsarbeit anderer re ist die spezifische Entwicklungsarbeit wichtig, d. h. die 1 g Embryo verbrauchte chemische Energie. Dividiert man Entwicklungsarbeit mit dem Gewichte der Trockensubstanz Embryo, so erhält man diesen Wert auf Trockensubstanz ogen. Für die Funduluslarven[1]) ergibt sich

$$\frac{0,723}{0,535} = 1,35 \text{ Cal., abgerundet } 1,4 \text{ Cal.}$$

Nun muß aber in Betracht gezogen werden, daß ein großer il des Gewichtes der ausgeschlüpften Larven auf den unbrauchten Dotter fällt, den sie noch mit sich führen. Ich be versucht, an Larven durch vorsichtiges Abpräparieren des tters das Verhältnis zu dem übrigen embryonalen Körper erhalten; es stellte sich heraus, daß etwas mehr als die lfte der ausgeschlüpften Larven aus Dotter besteht. Da Entwicklungsarbeit sich auf ausgebildete organisierte emonale Substanz bezieht, so erhöht sich die eben berechnete hl für die spezifische Entwicklungsarbeit auf etwas mehr als s Doppelte, d. h. auf etwa 3 Cal. (Berechnet man die spezische Entwicklungsarbeit der organischen Substanz, so erhält an 4 Cal.) Tangl hat für die spezifische Entwicklungsarbeit es Hühnerembryos 3,8 Cal. pro 1 g Trockensubstanz erhalten und sein Schüler K. Farkas beim Seidenspinner 3,1 Cal.

Nach meinen Versuchen ist also die spezifische Entwicklungsarbeit im Fundulusei — wenigstens für die embryonale Trockensubstanz — annähernd von der gleichen Größe wie im Hühner- und Seidenspinnerei. Meine Versuche sprechen demnach ebenfalls für die Hypothese Tangls, daß „die spezifische Entwicklungsarbeit der tierischen Organismen keine Funktion ihrer phylogenetischen Stellung und Organisation ist, und daß dementsprechend die embryogenetische Bildung verschieden hoch organisierter lebender tierischer Substanz mit gleichem Verbrauch an chemischer Energie erfolgen kann, und so weit bisher untersucht, auch tatsächlich erfolgt“.

---

[1]) Die spezifische Entwicklungsarbeit der frischen, wasserhaltigen ubstanz konnte ich nicht berechnen, weil das frische Gewicht der arven und Eier nicht ermittelt wurde.

Zum Schlusse möchte ich noch auf eine weitere Übereinstimmung zwischen Fundulus einerseits und Huhn- und Seidenspinner anderseits hinweisen.

Versucht man den spezifischen Energiegehalt — (Verbrennungswärme) — der während der embryonalen Entwicklung verbrauchten organischen Substanz zu berechnen, indem man die verschwundene chemische Energie mit der verbrauchten organischen Substanz dividiert, in meinen Versuchen.

$$\frac{723\ \text{cal}}{0{,}08\ \text{g}} = 9{,}0\ \text{Cal.},$$

so erhält man, wie die Rechnung zeigt, einen Wert, der dem Verbrennungswert des Fettes entspricht. Das spricht dafür, daß im Fundulusei sowie im Hühner- und Seidenspinnerei die chemische Energie des Fettes die Entwicklungsarbeit liefert, d. h. es wird während der Embryogenese hauptsächlich Fett verbrannt.

Ich habe noch den Energie- und Stoffverbrauch in den verschiedenen Stadien der Entwicklung des Fundulusembryo untersucht, um vor allem festzustellen, ob die Entwicklungsarbeit auch in den verschiedenen Stadien der Embryogenese die gleiche ist und von den gleichen Stoffen geliefert wird. Diese Untersuchungen müssen aber, wie schon erwähnt, noch ergänzt werden.

Die mitgeteilten Untersuchungen habe ich unter der Leitung des Herrn Prof. F. Tangl in der königl. ung. tierphysiologischen Versuchsstation in Budapest ausgeführt.

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Von

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ON THE ORIGIN OF DOUBLE-YOLKED EGGS.

OTTO GLASER.

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ON THE ORIGIN OF DOUBLE-YOLKED EGGS.

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INTRODUCTION.

By comparing five cases of double eggs that happened to fall into his hands, with a considerable number described in the literature, Parker ('06) was able to divide these abnormalities, on the basis of the factors probably involved in their production, into three classes: "First, those whose yolks have come from an abnormal ovary, but have passed through a normal oviduct; secondly, those whose yolks have come from a normal ovary, but have passed through an abnormal oviduct; and finally those produced by an ovary and oviduct, both of which have been abnormal in their action" (p. 17).

To the first group belong eggs which contain ordinarily two yolks, surrounded either by individual vitelline membranes or by a common one. The second class is made up of eggs in which normal yolks are imbedded in abnormal secondary envelopes, whereas the third is composed of cases in which one egg, consisting usually of shell, shell-membranes, albumen and a small yolk, is enclosed in an outer one of normal construction. Nine cases found in the literature by Parker, four additional ones studied by himself, as well as some of those referred to by Hargitt in a neglected paper ('99) and in a later one ('12), belong to this group.

In this article I shall report observations on a case belonging to the first class, and I do this, not because double-yolked eggs are sufficiently rare to warrant further description, but because I have been unable to find in the literature accessible to me any account of the ovarian abnormalities associated with the production of this type of egg. In fact from Parker (*loc. cit.*, p. 16) one gains the impression that these peculiarities are hardly marked enough to justify the use of the word "abnormal." Thus he says: "The laying of eggs with two yolks may become, as Landois ('78, p. 24) declares, almost habitual with certain answered by studying the others. Follicle *c* suggests that compound follicles might have originated by a process of budding. To test this view part of the wall of *c* together with the attached follicle *c''* was cut into sections 10μ thick. To my surprise no connection whatever between the lumina of the two follicles could be found. The place where this was expected showed the granulosa and fibrosa of both, and between the fibrosæ, a thick connective tissue mass highly vascularized. I examined other apparently branching follicles, such as *e*, and again found the same thing true. In certain other instances connections between adjacent follicles do exist, but these are not via the necks of the attached follicles, but through their sides, and must therefore have come about secondarily by the disappearance in these places not only of the inter-follicular tissue, but also of the granulosæ and fibrosæ. If *c''* then is not a bud, how did it come to occupy its present position?

In *d*, follicles *d''* and *d'''* are attached to the main suspensorium by short necks of their own. These however are so completely fused proximally with the suspensorium of *d* that their independence is largely obliterated. *E''*, although firmly fused to *e*, really has a suspensorium of its own, but this is reduced to an extremely fine thread, visible as an independent attachment only in one or two places. *C''* upon careful examination proves also to have indications of an independent attachment to the central ovarian mass. The threads therefore are reduced suspensoria, and the compound follicles are fusion products.

The question how these fusions occur remains to be answered. Indications as to how these could have come about are contained in the facts just discussed, for these suggest that one follicle is carried away from the central ovarian mass by another. If this is true, we should be able to discover various stages in the process. Accordingly we can interpret *b* as a late stage in which intimate union has occurred among the follicles distally, whereas proximally the suspensorium of the group shows the multiple nature of its origin by its distinct division into a number of strands. *A* is another late stage, only here the follicles seen are larger than in *b*, their fusion is less complete, and *a''* has worked its way into *a* so that the yolk of the latter came to lie between til June, 1911. She was a beautiful example of the breed, but d an unusually large comb. She was a loud and boisterous ckler, and always made a great fuss after laying. Apparently e never exhibited any tendency to set. Although it cannot stated with certainty that she never laid normal eggs, it is rtain that a very large percentage was abnormal.

The eggs during her last year measured on an average 7.5 cm. their long diameter, 4.5 cm. in their short. The yolks, always individual vitelline membranes, were equal in size with an erage diameter of 3 cm. They were always in contact, or actically so, and were surrounded by a common jelly mass.

The records are too meager to show whether there was any ythm in her laying. On occasion she laid double-yolked eggs two or even three successive days. Spells of hyperactivity ch as these were frequently followed by periods of indolence sting from one to several weeks. Sometimes she would lay ery other day for a period.

Although the eggs were always large they decreased in later ars. Even then laying caused difficulties as evidenced by ises suggestive of struggle. After a period of laying the bird arly always "moped," hanging her head and refusing to eat. hese sick spells were not noticed during the first year or two, though there was never any eagerness for food.

I have given these details because they may be valuable as inical symptoms, and because they show that the ovary, which, we shall see, was abnormal structurally, was so physiologically well. In fact I am inclined to think that the structural peculiarities of this organ are a consequence primarily of some physiogical defect, and that the abnormal spatial relations brought bout in this way gave the physical basis for the production of ouble-yolked eggs.

THE ANATOMY OF THE OVARY.

The ovary was removed immediately after the death of the ird and fixed in Zenker's fluid by my brother, R. W. Glaser. he gross appearance as well as numerous details are reproduced Fig. 1, which I made by first printing an outline of the organ hrough glass on blue-print paper, and after transferring this to ristle board, filling in the necessary minutiæ freehand.

they are quite small, like those on the necks of *c* and *j*, whereas at times they may be completely hidden, as in the neck of *a''*, where another follicle was discovered only after the dissection shown in Fig. 2 had been made.

Given weakness on the part of the tunica albuginea, three minor variations of one and the same process are capable of accounting for these different types of follicles. I have tried to represent this in a series of diagrams (Fig. 3).

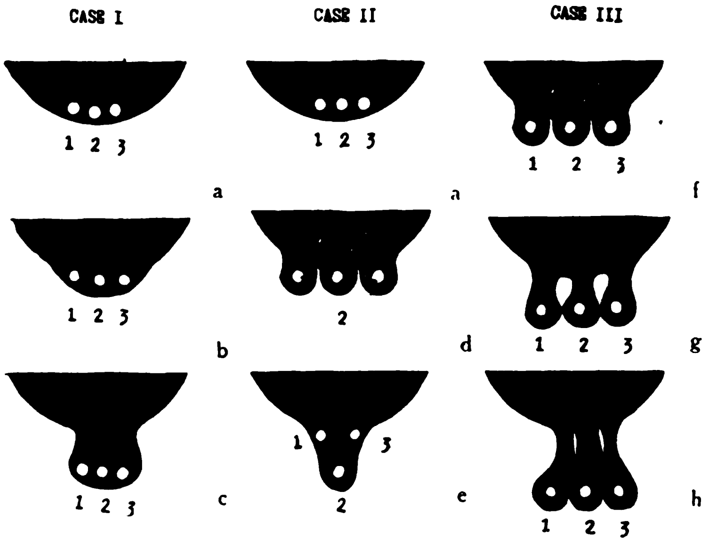

FIG. 3. Diagrams to illustrate the origin of compound follicles. Description in text, p. 182–183.

If we imagine that the ovarian surface evaginates rather broadly beneath egg 2, Case I., diagram *a*, then eggs 1 and 3, which are supposed to lie very close to 2, will be carried away from the ovary, as in *b* and *c*. This would lead to the formation of a follicle of the type *i* and *j*, Fig. 1.

If on the other hand only a very small evagination should take place under egg 2, Case II., its neighbors, 1 and 3, if carried away from the ovary, would not come to lie in such close proximity to 1 in the evagination. In fact they would lie higher up in the suspensorium, *d*, *e*, Fig. 3, and in this manner sufficiently great differences in early distribution would account for the later development of follicles at various points on the suspensoria as in *a*, *c*, *f*, *j*, etc., Fig. 1.

If, owing to greater distances between the eggs, or to greater stiffness on the part of the tunica, separate evaginations should occur beneath three follicles as in Case III., diagram *f*, Fig. 3, these if not too remote from one another might give rise to a triple follicle, having a common suspensorium except near the ovary where its multiple origin would still be apparent upon close examination. These processes, represented diagrammatically in *g* and *h*, would give rise to complexes similar to *a* and *b*, Fig. 1. If the number of follicles involved were greater, more complicated results would follow. The possibilities of greater complication are clearly indicated by a group of relatively young follicles such as *z*, Fig. 1, as well as by instances in which follicles, of patently distinct origin proximally, have fused distally with others with whom they were brought into contact secondarily by mere accident. Good examples of adhesions of this kind are follicles *x* and *y*, Fig. 1.

In its relation to the production of double-yolked eggs, this ovary is full of suggestions. Follicle *f*, Fig. 1, is an empty double one, and there can be little doubt that it was instrumental in the production of one of the abnormal eggs. Follicles *a* and a'' contained yolks of essentially the same size, and there seems to be no good reason for doubting that these two would have been shed at the same time. In *b*, the follicles fall into two groups of two each. In one of these they are distinctly larger than in the other, but the follicles within each group are of the same size. It seems likely that in this complex we have the physical basis for the production of two double-yolked eggs in rapid succession. *Z* suggests the possibility of a series of abnormal eggs such as this bird is known to have laid on several occasions.

PHYSIOLOGY OF THE OVARY.

The morphological findings and their interpretation leave open the question as to how one may picture the physiology of this organ. Follicle *b*, Fig. 1, demonstrates the possibility of relatively independent growth on the part of two groups of eggs, as well as the possibility of synchronism in eggs belonging to the same group. How this can be is answerable by assuming that the vascular supply to the two groups was different, whereas to the individual members of each group it was practically the same. The physical basis for this might easily be found in the character of the fusion undergone by the individual suspensoria, for if this should be such as to partially occlude the flow of blood in certain directions, the follicles supplied by the vessels involved would lag behind those furnished with a better supply. That the blood supply to certain follicles must be affected, is clearly shown by the suspensoria which are reduced to mere threads.

In certain instances, however, a follicle despite its reduced suspensorium is practically as large as the neighbor to which it is attached, as in the case of *e''*. This suggests the establishment of a secondary blood supply derived from the better equipped follicle. The highly vascular condition of the connective tissue between the granulosæ certainly is favorable to this interpretation. In fact in view of the structural relations it is difficult to see how the blood supply could be otherwise than identical in this case.

Identity of vascularization makes another fact understandable. A follicle such as *c''* would under normal conditions have a blood supply much less than that of its "host" *c*. As it probably is dependent on that of *c*, however, owing to its intimate fusion with it, and the reduction to almost nothing of its own suspensorium, it is likely to have more material brought to it than it can metabolize in normal fashion. This follicle, as well as others of its size similarly placed, showed considerable depositions of yolk in the lacunæ of their hypertrophied granulosæ.

This fact is not only suggestive as to the rôle played by the granulosa during oögenesis, but also of the part of the egg itself in initiating the deposition of yolk, for if the ovum in this instance is not supposed to have been less permeable to the raw materials of yolk than the one in the larger follicle, where only traces of yolk could be found in the granulosa, it is difficult to see why the two eggs should have been unequal in size. There must be a period in the ovarian life of an egg when its permeability to certain substances is suddenly greatly increased. Riddle's work ('11) suggests the same thought, but this abnormal ovary ndicates that the conditions followed by increased permeability re to be looked for in the egg. If ova in the same state have an lentical blood supply they will undergo equivalent growth, but nere identity in their circulation does not insure this.

Although these considerations are necessarily speculative, their ossible relation to more remote matters may be briefly mentioned. It is certainly a remarkable fact that in the human vary, to mention a case among mammals, and in the hen's vary, to mention one among birds, normally one egg ripens after nother, whereas in the ovaries of amphibians and fishes thouands of eggs may ripen at one time. It is quite possible that ne conditions which in the one case lead to an abnormality are lentical with those which in the other give a typical result. n no case do all the eggs contained in any of these ovaries ripen t the same time. This certainly points to alterations in the ermeability of the ovum as a factor in the initiation of its rowth period, and suggests that these changes are directly raceable to the activity of the egg. The fusion of normally istinct follicles in the hen's ovary brings about secondarily an lentical vascularization and thus accidentally duplicates the utritive conditions prevalent in the ovaries of certain other orms.

Identity of blood supply then is suggested as an explanation f the synchronic yolk formation in these eggs, whereas a change n permeability undergone independently on the part of the ggs themselves introduces this period of growth. This change n permeability must necessarily also be synchronic if doubleolked eggs are to be produced, but how this synchronism is rought about in certain eggs, and how it is prevented in others, annot be profitably discussed in our present state of knowledge.

The important thing after all is that a bird's ovary, with ollicles secondarily fused as described in the preceding pages, does actually give rise to a series of double-yolked eggs, and this remains true even if my suggestions as to the development and physiology of this organ should prove to be entirely wrong. It is to be hoped that the clinical symptoms given may aid in the discovery of further material upon which more detailed studies in the living state could be carried out with great advantage to our knowledge of the more obscure points in the physiology of egg-production.

Zoölogical Laboratory,
University of Michigan,
November 22, 1912.

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ON INDUCING DEVELOPMENT IN THE SEA-URCHIN (ARBACIA PUNCTULATA), TOGETHER WITH CONSIDERATIONS ON THE INITIATORY EFFECT OF FERTILIZATION

OTTO Charles GLASER

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ON INDUCING DEVELOPMENT IN THE SEA-URCHIN (ARBACIA PUNCTULATA), TOGETHER WITH CONSIDERATIONS ON THE INITIATORY EFFECT OF FERTILIZATION[1]

I. THE INITIATION OF DEVELOPMENT WITH DILUTE SEA WATER

IN the course of work on the energetics of development, it became necessary to study in detail the question of water absorption at various stages of embryogeny. For certain phases of these studies the eggs of *Arbacia punctulata* proved extremely favorable. In various concentrations of sea water these eggs behave exactly as expected, but in 25 per cent. sea water (25 c.c. sea water + 75 c.c. H_2O dist.) fertilization membranes appear. The process takes place in from one to one and a half minutes at ordinary temperatures. In two minutes many eggs as well as their nuclei are cytolized, and in three minutes this is true of most of the eggs.

The membrane in question is a true fertilization membrane, and if at the proper moment the eggs are brought back into normal sea water, or better still, hypertonic sea water (50 c.c. sea water + 8 c.c. 2.5 N NaCl), cleavage takes place. Since July 18 I have succeeded in rearing a considerable number of ciliated larvæ.

II. THE INITIATION OF DEVELOPMENT WITH EGG EXTRACT

If fresh ovaries of *Arbacia* are ground up in a mortar with pulverized glass and a small quantity of sea water, the liquid, when filtered, has a color not unlike that of blood serum. This fluid, if allowed to act on ripe eggs contained in an equal quantity of sea water, proves to be an excellent initiatory agent, for if the eggs after one to two hours are placed in normal sea water, many divide, although no fertilization membrane appears.

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[1] Preliminary communication.

III. THE THEORY OF INITIATION, PARTHENOGENETIC METHODS AND THE FERTILIZATION MEMBRANE

It is well known that development can be induced in many kinds of eggs by very diverse means—lipoid solvents, increased osmotic pressure of the surrounding medium, electricity, heat, cold, mechanical shock and even pricking the egg surface, have all proved effective in one case or another, but so far as I am aware the use of egg extract from the same species is new, as well as the production of genuine fertilization membranes in *Arbacia punctulata* by means of dilute sea water. In one of the California sea urchins, Loeb[3] has reported the formation of membranes after the addition of distilled water, but from certain details it seems that the fertilization membrane of at least one of the California urchins resembles that of *Asterias forbesii,* and this differs quite markedly from that of *Arbacia punctulata.*

Loeb,[3] on the basis of his own investigations and those of others, has formulated a theory on the initiation of development which for normal fertilization and certain of the parthenogenetic methods, postulates (*a*) an increased permeability of the ovum due to the action of lipoid solvents or hæmolytic agents; (*b*) the formation of a fertilization membrane in consequence of this superficial cytolysis.

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[3] Loeb, Jacques, "Die chemische Entwicklungserregung, etc.," Julius Springer, Berlin, 1909.

Of an increase in permeability synchronous with the initiation of development there is not the slightest doubt, although the great variety of parthenogenetic methods long ago indicated that permeability is increased, in other ways than by action on surface lipoids. With the employment of some parthenogenetic methods, fertilization membranes appear, with others, not, and even the employment of lipoid solvents themselves may or may not be followed by the appearance of a fertilization membrane. One and the same egg, as in the present case, may be induced to develop with or without the appearance of such a membrane.

IV. EXPERIMENTAL ANALYSIS OF THE FERTILIZATION MEMBRANE

According to Kite's[3] dissection, the egg of *Arbacia* has a vitelline membrane tightly glued to its surface. Outside this is a thin jelly. The appearance of the fertilization membrane, according to this description, is due to the swelling of the vitelline membrane, and the formation of a phase boundary between it and the thin outer jelly.

This description I believe to be essentially correct for the following reasons:

1. The fertilization membrane also has an inner visible boundary. In certain localities of the two- and four-cell stage this inner surface of the fertilization membrane is plainly visible, has indeed been often figured and I believe misinterpreted. In the stages in question a narrow perivitelline space can be seen around the egg, but the fertilization membrane adheres to the egg surface here and there by strands. As a consequence, when the egg divides, some of these strands are drawn down between the cleavage cells, and as certain portions of the surface of these are

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[3] Kite, G. L., "The Nature of the Fertilization Membrane, etc.," SCIENCE, Vol. XXXVI.

further removed from the fertilization membrane than the original egg, the inner limit of this membrane, as well as the perivitelline space itself, becomes visible. The perivitelline space seems to be identical with the so-called "hyaline plasma-layer," and homologous with the perivitelline space of the fertilized starfish egg.

2. By means of hypertonic solutions as well as by extract of themselves, sea-urchin eggs can be induced to divide without the appearance of a fertilization membrane. Development, however, does not proceed normally because the blastomeres fall apart. Since the vitelline membrane is tightly glued to the surface of this egg and a perivitelline space appears after the membrane has swollen, eggs dividing without the formation of this space have the membrane adhering to the resulting blastomeres. In consequence, these cells, instead of being in intimate contact with one another as they normally are, are each enclosed in a separate vitelline membrane. In other words, when the vitelline membrane is not lifted off the egg surface, it divides with the egg, which is what one would expect. If this idea is correct, cleavage cells which have originated by the division of an egg without a "fertilization" membrane should be able to "form" such membranes under suitable conditions, and this I have observed. Immersed in dilute sea water, isolated cleavage cells, derived from ova which have not formed "fertilization" membranes, form them in from one to two minutes.

3. Egg fragments can also be produced by shaking. No fertilization membranes appear in such eggs or their fragments as the result of the mechanical agitation, but when treated with dilute sea water or sperm, membranes appear in some of the fragments, but not in others. Both kinds of fragments have been fertilized with sperm and allowed to develop,

some with and some without the membrane. This result can only be understood if we accept Kite's discovery that the fertilization membrane in *Arbacia punctulata* appears when a preexisting jelly, closely adherent to the surface of the egg, swells and changes its optical properties.

4. From the above experiments one may infer that a fertilization membrane may appear around part of an egg, instead of the whole. If Kite's jelly is ruptured the egg flows partially through the hole in the membrane, and assumes a dumbbell shape. If it is now fertilized with sperm, or treated with dilute sea water, a fertilization membrane appears on one sphere of the dumbbell, but not on the other. Such eggs are capable of development.

5. The appearance of a fertilization membrane in *Arbacia punctulata* is not a function of the living egg, for if the egg is crushed, or even dried completely in a desiccator for days, membranes still appear after proper treatment.

V. WHAT MAKES THE FERTILIZATION MEMBRANE APPEAR NORMALLY?

If the interpretation given to the results outlined is correct for *Arbacia punctulata,* it is easy to see why the fertilization membrane should appear in dilute sea water, or in distilled water. But why does it appear under normal conditions in sea water?

The exact mechanism of the process is not yet clear, but it seems to be a function of the number of sperm present. If one inseminates eggs very carefully so that not more than four or five spermatozoa come into contact with each one, the fertilization membrane does not appear. I have repeated this experiment many times and have controlled it by the most careful observations with different powers on fresh material as well as stained.

Such preparations show sperm plainly adhering to Kite's jelly in every egg, but the "membrane" does not appear. Eggs treated in this manner do not develop, although some of the smaller ones may form asters. What it is in the sperm that brings about the swelling of the jelly has not yet been determined. However, beautiful fertilization membranes may be caused to appear in two to three hours by treating the eggs with minute infusoria. No membranes appear in the controls, nor do the eggs whose membranes have appeared develop when returned to sea water. Three possibilities suggest themselves—an acid effect, a mechanical effect or a heat effect. No decisive experiment has as yet been devised.

These experiments suggest that in *Arbacia punctulata* the membrane swells before the sperm enters the egg, and not after. Experiments also show that when the phase boundary between Kite's jelly and the outer jelly is complete, sperm do not readily penetrate the fertilization membrane. From this it follows that the penetration occurs at the moment when the jelly is softened and begins to swell. Accordingly, eggs whose jelly has been partially softened by heat or infusoria should be capable of fertilization with small doses of sperm. This has actually been observed in a number of instances. The opposite experiment of hardening the jelly with Ca has been performed. Such eggs are extremely difficult and in many cases impossible to fertilize as the sperm do not stick.

VI. THE RELATION BETWEEN FERTILIZATION AND THE FERTILIZATION MEMBRANE

The relation between the initiation of development and the fertilization membrane in *Arbacia punctulata* is one of association rather than "causal," for the membrane may be made to appear without development, and development may be initiated without the appearance of the membrane. In ***Asterias forbesii*** the association is somewhat different, and so intimate that any method which causes the membrane to appear is at the same time a method of initiation provided the violence is not too great and the egg is in good condition and in a suitable medium. The explanation is simple. In ***Asterias*** the fertilization membrane does not depend on the swelling of a formed jelly, but instead, the egg peels itself away from the inner surface of a thin preexisting membrane. This peeling away seems to depend, not upon changes in the fertilization membrane, but upon changes in the surface film of the egg. When this is rendered more permeable, material leaves the egg and the egg shrinks away from its closely adherent covering which thus becomes visible. The perivitelline space in the starfish egg is homologous with that of the sea urchin egg, but is much larger.

The type of fertilization membrane found in *Arbacia punctulata* may be called hydrophilous, that of the starfish, ***Asterias forbesii,*** anhydrophilous.

VII. ON THE LOSS OF SUBSTANCES BY THE EGG AND THEIR NATURE

The starfish egg upon peeling off from its anhydrophilous fertilization membrane is markedly smaller in volume than before. The same thing is true of *Arbacia*. Exact measurements will be given when I publish exact details of these experiments. No doubt much of the material lost by the egg is water. F. R. Lillie[4] in a series of fundamental researches has shown that the fluid over-fertilized eggs may contain at the least two classes of substances, (*a*) "iso-agglutinins" and (*b*) a substance having a chemotactic influence on the sperm. From Elder's[8] investigations as well as certain observations of my own, it appears possible that the chemotactic substance is contained in the outer jelly of the *Arbacia* egg. I have been able to verify the " iso-agglutinin " and its effects as described by Lillie in the case of *Arbacia* and *Asterias.*

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[4] Lillie, F. R., "Studies of Fertilization," I. and II., *Jour. of Morph.*, Vol. 22; III. and IV., *Jour. of Exp. Zool.*, Vol. 12; V., *Jour. of Exp. Zool.*, Vol. 14.

Ovarian extract of *Arbacia,* when present in sufficient quantities, retards the development of normally fertilized *Arbacia* eggs. If the extract is added to blastulæ which have developed in normal sea water, these are instantly slowed down and absorb water. *Arenicola* larvæ also have their permeability increased by the *Arbacia* extract, as can be very prettily seen by their loss of pigment. They also slow down in their movements and are slightly and reversibly agglutinated.

These observations suggest that the ovarian extract, as well as the secretions of the egg on fertilization contain substances that not only influence permeability, but may reduce the oxidations in the cell.

VIII. THE THEORY OF INITIATION

The theory of initiation, as given by Loeb, postulates essentially that initiatory influences place the egg in a condition in which its oxidative processes can proceed, or proceed normally. This is accomplished by increasing the permeability of the egg, and in the case of many parthenogenetic agents, as well as in normal fertilization by sperm, the permeability change may be brought about by lipoid solvents. The fertilization membrane may or may not appear after the use of lipoid solvents, and when, as in the case of the starfish egg, it does appear, it may also be made to do so with any other method of increasing the permeability of the plasma film. These facts, many of which have been emphasized by Loeb,[5] R. S. Lillie[6] and others, by no means prove that the theory of initiation is wrong. Indeed, they are all in harmony with this view if we remember that an hydrophilous fertilization membrane may or may not appear, depending on circumstances, whereas an anhydrophilous one like that of *Asterias* is certain to appear when, as the result of a permeability change, the egg shrinks away from its enclosing capsule.

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[8] Elder, J. C., "The Relation of the Zona Pellucida to the Formation of the Fertilization Membrane," *Arch. f. Entwicklungsmechanik,* Vol. 36.

How can increased permeability initiate development?

The ovum demonstrably has the necessary mechanism to undergo development of itself. It is a cell with a long metabolic history and before development is initiated its plasma film is relatively impermeable. This may involve the accumulation of "waste" products, and these we may believe to automatically inhibit further metabolic processes. Loeb has shown that these processes are oxidations, and my experiments show that substances can be extracted from the eggs which reduce the rate of development and have a marked effect in decreasing the activity of *Arbacia* as well as *Arenicola* larvæ. It does not seem unreasonable to suppose therefore that these materials are active because they reduce oxidations. The mere fact that they also increase cell permeability and are good initiatory agents is beside the point, for increased permeability in *Arenicola* larvæ is also associated with acceleration of movement.

One may extend the theory of initiation and assume that all agencies that initiate development do so because through increased permeability of the plasma film the egg is enabled to loose substances antagonistic to oxidation. By freeing itself of these inhib-

[6] Lillie, R. S., "The Physiology of Cell Division," *Jour. of Morph.*, Vol. 22.

itors, a chemical equilibrium is disturbed, and oxidation, and with it development, is free to go on.

In this way we can explain why a mature starfish egg, if unfertilized, may oxidize itself to death, for we may suppose that its permeability has been sufficiently increased by maturation to accelerate oxidation, but not enough to initiate development proper. We can also bring all parthenogenetic methods whatsoever, as well as normal fertilization, under a common point of view, for the increased permeability, no matter whether produced by electricity, heat, cold, mechanical shock, specific chemical alteration of the membrane, lipoid solvents, or pricking, is all that is necessary to enable the egg to free itself from its accumulated inhibitors. Why the egg should develop after treatment with hypertonic solutions is also clear, for if in such media the plasma film is permeable to the inhibitors, loss of water by the egg would, directly or indirectly, accelerate the loss of antagonists. That these are lost in hypertonic sea water is shown by special experiments.

In conclusion, I must thank my colleague, Dr. W. E. Garrey, who kindly allowed me to demonstrate to him various steps in the investigation, and to whom I am indebted for a number of valuable suggestions and criticisms.

OTTO GLASER

WOODS HOLE, MASS.,
August 4, 1913

PAMPHLET COLLECTION

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THE CHANGE IN VOLUME OF ARBACIA AND ASTERIAS EGGS AT FERTILIZATION.

OTTO Charles GLASER

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THE CHANGE IN VOLUME OF ARBACIA AND ASTERIAS EGGS AT FERTILIZATION.[1]

OTTO GLASER.

In my paper on the initiation of development, Glaser (13), I stated that the eggs of *Arbacia punctulata* and *Asterias forbesii* are smaller in volume after fertilization than before. In the pages that follow I wish to present the evidence for this assertion in some detail, since for the present at least, it must stand alone. *Strongylocentrotus* (*purpuratus?*) shows no recognizable loss of volume according to Loeb ('08) but this result does not contradict mine. The loss depends on several conditions, and in *Asterias* is much greater than in *Arbacia*. This suggests the possibility of forms in which it may easily be too small to measure. The measurements of McClendon ('10), also, do not contradict mine, although they seem to show that the very eggs I worked with, on fertilization lose in a molar solution of dextrose, but remain constant, or even gain a little in sea water. The evidence consists in the determination of the diameters of 19, 20, 10 and 11 eggs respectively. The great variability of the ova of *Arbacia punctulata*, however, proves that reliable results can be gotten from a small number of eggs, only when the identical ones are measured before and after fertilization.

Careful tracings were made at known magnifications with the aid of a camera lucida, and a crow-quill pen, on paper of known weight. On the assumption that the eggs are spheres, and that one is really drawing their great circles, the areas of the outlines obtained in this manner, were first determined by means of a planimeter, and afterwards by carefully cutting them out and weighing the discs. For enlargements of 740 diameters both methods gave identical results, but for the smaller magnifications the method of weighing proved more reliable. For this reason, in the comparisons of fertilized with unfertilized eggs, only data obtained by the latter method are reported, whereas the variability curves are based on factored measurements by the planimeter. The same curves were first constructed from the original measurements.

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[1] From the Marine Biological Laboratory at Woods Hole, and Zoölogical Laboratory of the University of Michigan.

ON THE CONSTANCY IN VOLUME OF THE EGGS OF A SINGLE FEMALE.

In Table I. are given the diameters of 280 different eggs derived from one female, measured in groups of ten, at three distinct periods after removal from the ovaries. The results show not only a remarkable constancy for the ova of a single individual, when grouped in this manner, but what is of even more immediate importance, they show that in four hours, the eggs do not undergo any change in volume discoverable by the methods employed.

TABLE I.

FEMALE B.

10 eggs, 4.35 P.M. 81.4 micra.	5.40 P.M. 82.6 micra.	8.35 P.M. 79.6 micra.
10 eggs, 4.35 P.M. 80.0 micra.	5.40 P.M. 80.8 micra.	8.35 P.M. 81.6 micra.
10 eggs, 4.35 P.M. 81.2 micra.	5.40 P.M. 80.8 micra.	8.35 P.M. 79.6 micra.
10 eggs, 4.35 P.M. 80.6 micra.	5.40 P.M. 79.4 micra.	8.35 P.M. 79.0 micra.
10 eggs, 4.35 P.M. 82.4 micra.	5.40 P.M. 78.6 micra.	8.35 P.M. 81.0 micra.
10 eggs, 4.35 P.M. 80.6 micra.	5.40 P.M. 81.6 micra.	8.35 P.M. 82.4 micra.
10 eggs, 4.35 P.M. 78.8 micra.	5.40 P.M. 81.2 micra.	8.35 P.M. 82.2 micra.
10 eggs, 4.35 P.M. 81.4 micra.	5.40 P.M. 80.8 micra.	8.35 P.M. 81.0 micra.
10 eggs, 4.35 P.M. 78.2 micra.	5.40 P.M. 77.4 micra.	8.35 P.M. 80.0 micra.
10 eggs, 4.35 P.M. 83.6 micra.	5.40 P.M. micra.	8.35 P.M. micra.
Average 80.8 micra.	Average 80.4 micra.	Average 80.6 micra.

ON THE VOLUME OF THE EGGS OF DIFFERENT FEMALES.

To what extent the average volume of the ova of different females is constant, I am unable to say, since scarcely anything much less than one hundred eggs from each would suffice to decide the question. One would not expect great constancy however unless the numerous factors entering into the result were identical. In the one case in which I have the requisite number of measurements for such comparison, the average diameter is the same as that found for female B.

TABLE II.

FEMALE C.

10 eggs 80.4 diam.
10 eggs 82.2 diam.
10 eggs 80.2 diam.
10 eggs 79.6 diam.
10 eggs 81.6 diam.
10 eggs 79.0 diam.
10 eggs 77.8 diam.
10 eggs 79.2 diam.
10 eggs 79.2 diam.
Average 80.0 diam.

THE DIAMETERS OF INDIVIDUAL EGGS.

In the two preceding tables the measurements are given for groups of ten eggs each. Comparison of the various sets indicates considerable variability, but the true extent of this can only be realized when a large number of ova is treated individually. This was done with 100 eggs of female B and 100 of female C. The results are given in Tables III. and IV.

TABLE III.

FEMALE B, INDIVIDUAL EGGS.

Of 100 eggs 1 had a diameter of	49.8 micra.	
Of 100 eggs 1 had a diameter of	58.8 micra.	Average of 3 = 57.1
Of 100 eggs 1 had a diameter of	61.6 micra.	
Of 100 eggs 1 had a diameter of	64.2 micra.	
Of 100 eggs 1 had a diameter of	65.4 micra.	Average of 3 = 65.5
Of 100 eggs 1 had a diameter of	66.8 micra.	
Of 100 eggs 2 had a diameter of	68.0 micra.	
Of 100 eggs 4 had a diameter of	70.6 micra.	Average of 11 = 70.8
Of 100 eggs 5 had a diameter of	72.0 micra.	
Of 100 eggs 5 had a diameter of	73.2 micra.	
Of 100 eggs 3 had a diameter of	74.6 micra.	Average of 16 = 74.2
Of 100 eggs 8 had a diameter of	76.0 micra.	
Of 100 eggs 3 had a diameter of	77.2 micra.	
Of 100 eggs 5 had a diameter of	79.6 micra.	Average of 15 = 79.2
Of 100 eggs 7 had a diameter of	79.8 micra.	
Of 100 eggs 8 had a diameter of	81.2 micra.	
Of 100 eggs 2 had a diameter of	82.4 micra.	Average of 20 = 82.6
Of 100 eggs 10 had a diameter of	83.8 micra.	
Of 100 eggs 7 had a diameter of	85.0 micra.	
Of 100 eggs 4 had a diameter of	86.4 micra.	Average of 14 = 84.5
Of 100 eggs 3 had a diameter of	87.6 micra.	
Of 100 eggs 6 had a diameter of	89.0 micra.	

TABLE III. *Continued.*

FEMALE B. INDIVIDUAL EGGS.

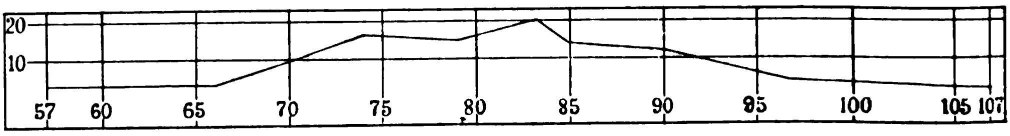
Of 100 eggs 3 had a diameter of 90.4 micra.	Average of 12 = 90.0
Of 100 eggs 3 had a diameter of 91.6 micra.	
Of 100 eggs 2 had a diameter of 94.2 micra.	
Of 100 eggs 1 had a diameter of 96.8 micra.	Average of 4 = 96.5
Of 100 eggs 1 had a diameter of 100.8 micra.	
Of 100 eggs 1 had a diameter of 102.0 micra.	
Of 100 eggs 1 had a diameter of 112.6 micra.	Average of 2 = 107.3

TABLE IV.

FEMALE C, INDIVIDUAL EGGS.

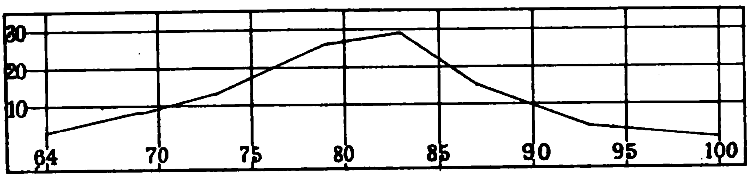
Of 100 eggs 1 had a diameter of 59.4 micra.	
Of 100 eggs 1 had a diameter of 65.2 micra.	Average of 3 = 63.7
Of 100 eggs 1 had a diameter of 66.6 micra.	
Of 100 eggs 2 had a diameter of 68.0 micra.	
Of 100 eggs 5 had a diameter of 69.6 micra.	Average of 8 = 69.4
Of 100 eggs 1 had a diameter of 71.0 micra.	
Of 100 eggs 5 had a diameter of 72.2 micra.	
Of 100 eggs 4 had a diameter of 73.8 micra.	Average of 13 = 72.9
Of 100 eggs 4 had a diameter of 75.4 micra.	
Of 100 eggs 6 had a diameter of 76.8 micra.	
Of 100 eggs 9 had a diameter of 78.2 micra.	Average of 26 = 78.5
Of 100 eggs 11 had a diameter of 79.6 micra.	
Of 100 eggs 8 had a diameter of 81.0 micra.	
Of 100 eggs 11 had a diameter of 82.6 micra.	Average of 29 = 82.6
Of 100 eggs 10 had a diameter of 83.8 micra.	
Of 100 eggs 7 had a diameter of 85.6 micra.	
Of 100 eggs 4 had a diameter of 87.0 micra.	Average of 15 = 86.7
Of 100 eggs 4 had a diameter of 88.4 micra.	
Of 100 eggs 2 had a diameter of 89.8 micra.	
Of 100 eggs 1 had a diameter of 94.2 micra.	Average of 4 = 92.4
Of 100 eggs 1 had a diameter of 95.6 micra.	
Of 100 eggs 1 had a diameter of 100.0 micra.	Average of 1 = 100.0

If one treats the diameters and eggs in groups of three, and plots the latter on the ordinate and the former on the abscissa the following curve emerges for female B.



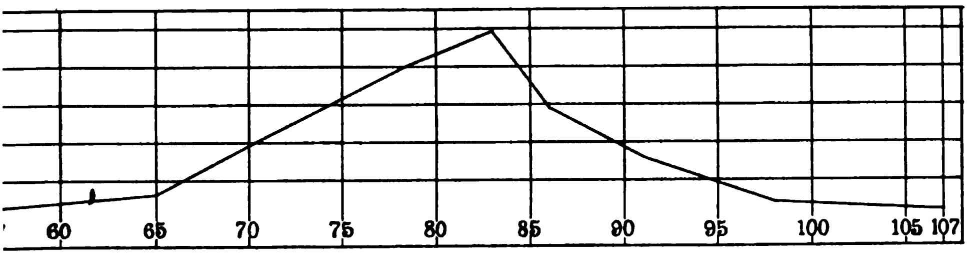
CURVE B. Diameters of 100 eggs from one specimen of ***Arbacia punctulata.***

The data from female C, treated similarly, give curve C.



CURVE C. Diameters of 100 eggs from one specimen of *Arbacia punctulata.*

Since the diameters for females B and C on the average are equal, the eggs of these two may be considered as homogeneous material. If this is done, then after the usual statistical simplifications,[1] an almost symmetrical curve results.



URVE BC. A composite of the diameters of 200 eggs from two specimens of *Arbacia punctulata.*

COMPARISON OF UNFERTILIZED AND FERTILIZED OVA.

The variability exhibited by these eggs and the differences in range between different females, show clearly that if one wishes to make trustworthy comparisons between the volumes of unfertilized and fertilized ova, it is either necessary to use a large number of each kind from a single female or to deal with a smaller number and measure the identical eggs before and after fertilization.

Before adopting the second alternative, I made a series of tracings of unfertilized and fertilized ova to see whether measureable differences really existed. The results, averaged, were as follows:

Unfertilized eggs; 77 measurements; average diameter, 74.1 micra.
Fertilized eggs; 91 measurements; average diameter, 70.7 micra.
Loss 3.4 micra.

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[1] The eggs are treated in sets of three, and average diameters not more than three micra apart are considered as belonging to the same group.

This result, further discussion of which is unnecessary, encouraged me to undertake a more careful study of a few eggs. Although involving more labor, this alternative was chosen in preference to the "wholesale" method, because the behavior of individual eggs could be definitely recorded. The probable advantage of this was indicated by the discovery by Glaser ('13) that the number of sperm used in insemination plays a rôle in the result. Accordingly "wholesale" measurements, which are satisfactory enough to decide whether or not a determinable change in volume occurs, have the disadvantage of covering up the fact that the loss is by no means a constant quantity.

In making these particular measurements, special precautions were taken. The eggs were traced one at a time, no other eggs were on the slide, and the focus before and after fertilization remained unchanged. This was not easy to accomplish, for the sperm are apt to set the egg in motion and change its position. Whenever this occurred, the attempt to secure the second measurement was abandoned. Naturally only a small number of cases could be collected under these conditions.

In order to fulfill the aforesaid requirements, and keep the magnification of the tracings absolutely constant, it was necessary to use small quantities of sperm. This however introduced other difficulties. The appearance of the fertilization membrane is the only convenient index of impregnation, but the membrane does not appear unless the spermatozoa are present in sufficient numbers. As a consequence, I often failed to observe the only available indication of fertilization.

While the results which I have seem to me to point in but one direction, the changes in volumes registered are in most cases probably minimal, for they depend on the amount of surface alteration, and this in turn is, within limits, a function of the number of sperm involved. Indeed with great excesses of sperm I have not infrequently observed a destructive alteration of the surface of the egg. This is more easily observable in *Asterias* than in *Arbacia*. It appears likely therefore that normally, or perhaps better, frequently, greater changes in volume occur than I have recorded.

The results on individual eggs dealt with singly in the manner described are given in the subjoined table.

TABLE V.

INDIVIDUAL EGGS BEFORE AND AFTER FERTILIZATION.

Female.	Egg.	Measurements.	Before.	Measurements.	After.	Change in diameter.
D_1	1	3	81.2	2	75.4	−5.8
D_1	2	3	80.6	3	68.8	−11.8
D_1	3	3	73.4	3	65.4	−8.0
D_1	4	3	78.8	3	72.8	−6.0
D_1	5	3	74.6	3	68.4	−6.2
D_2	1	4	70.0	3	66.6	−3.4
D_2	2	4	71.2	4	67.2	−4.0
D_2	3*	3	78.0	4	78.2	+0.2*
D_2	4	4	75.2	3	73.4	−1.8
D_2	5	3	78.0	4	75.2	−2.8
E	1	3	70.0	3	68.2	−1.8
E	2*	4	65.2	2	65.8	+0.6*
E	3	3	72.2	4	65.6	−6.6
E	4	3	72.2	4	69.4	−2.8
E	5	3	68.8	4	67.8	−1.0

With the exception of the starred eggs, D_2 3, and E 2, which within the limits of error may easily be considered constant, all the eggs showed a noticeable decrease.

The fact brought out by these measurements constitutes an additional support for the conception that an increase in the permeability of the egg accompanies fertilization. With such increase, other conditions remaining constant, one would expect a change in volume. The fact that unfertilized eggs, under certain conditions, may remain unaltered for hours, whereas there is a noticeable decrease in their diameters immediately after fertilization, strongly emphasizes the change that takes place.

COMPARISON OF UNFERTILIZED AND FERTILIZED OVA OF *Asterias*.

The results obtained in the case of *Arbacia* are more strikingly illustrated by a control observation made on the ova of *Asterias forbesii*. The measurements were made on eggs which had been removed from the ovaries and allowed to stand one hour in sea water in order to complete maturation. Tracings were made of two small groups from two females and these groups were then inseminated. It is impossible to identify individual eggs in this case, but this does not invalidate the result, since no eggs were either added or removed upon the addition of the sperm. Seventeen eggs derived from one female, and eight from the other were used, and the same eggs were traced after fertilization that had been traced before. The result was:

Female 1; 17 eggs	Diameter before fertilization, 103.6 micra. Diameter after fertilization, 85.2 micra.	Loss 18.4 micra.
Female 2: 8 eggs	Diameter before fertilization, 105.0 micra. Diameter after fertilization, 94.0 micra.	Loss 11.0 micra.

These values cannot be considered standard figures any more than those found in the case of the sea urchin, yet they seem to indicate with certainty that the egg of the starfish also loses in volume on fertilization. In fact the loss recorded is much greater than that found for *Arbacia* eggs. A portion of this difference may be due to unequal amounts of sperm used in the two cases, but the entire variation can hardly be accounted for on this basis. Normally the perivitelline space of fertilized *Asterias* eggs is proportionately larger than in *Arbacia*. If the space in question comes about as Loeb ('08) has contended, when a colloidal substance secreted by the egg absorbs water, then on the assumption that this material has essentially the same properties in *Arbacia* and in *Asterias*, the egg having the greater vitelline space should secrete the greater quantity, and therefore decrease correspondingly more in volume. That the *Asterias* eggs measured lost more on the average than the *Arbacia* treated either en masse, or as individuals, can be seen by a glance at the tables. One is led to suspect from these considerations that the surface of the *Asterias* egg is more easily modifiable than that of the *Arbacia* ovum. This is in harmony with the well known experience that artificial parthenogenesis can be induced in *Asterias* by a greater variety of methods than in *Arbacia*.

University of Michigan,
November 7, 1913.

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PAMPHLET COLLECTION

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12

A QUALITATIVE ANALYSIS OF THE EGG-SECRETIONS AND EXTRACTS OF ARBACIA AND ASTERIAS.

OTTO GLASER.

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INTRODUCTION.

Although it must have been seen earlier, F. R. Lillie ('12) was the first, so far as I know, to throw emphasis on the observation that when unfertilized *Arbacia* eggs are allowed to stand in a small quantity of sea water, the supernatant liquid takes on a reddish-brown color. The amount of discoloration varies directly as the number of eggs present, the amount of sea water used, and within limits, the time of exposure. If the eggs are now precipitated with the centrifuge, and the fluid above is decanted and filtered through paper, or simply decanted without filtration, it can be very easily shown that substances are present which do not occur in ordinary sea water.

I. ANALYSIS BY MEANS OF SPERM-SUSPENSIONS.

The best method of analysis is that discovered by F. R. Lillie, and although it was not my primary purpose, I have verified a number of his results ('12[1], '13[1], '13[2]). Following Lillie, I shall present my material under four heads: (*A*) Activation; (*B*) Chemotaxis; (*C*) Agglutination; (*D*) Paralysis.

A. ACTIVATION.

1. *Iso-Activation.*—As Lillie has pointed out, the phenomena of activation are presented with unequal clearness in different forms. The spermatozoa of *Nereis* for instance are so active in sea water that the effect of the egg-secretion is obscured. With *Arbacia*, the sperm, as is well known, are also active in sea-water, nevertheless a noticeable increase in the rate of their movements can be observed after the addition of a drop of the egg-secretion. *Asterias forbesii* is really much more favorable than either *Nereis* or *Arbacia* for the detection of the activating effect, for here the

spermatozoa are almost motionless. The suspensions I worked with showed only isolated spermatozoa executing occasional spasmodic movements. Upon the addition of the secretion from its corresponding egg, the *Asterias* sperm are thrown into violent activity contrasting sharply with their original state of quiescence.

2. *Hetero-Activation.*—Essentially the same phenomenon described under the head of iso-activation can be observed if *Asterias* sperm are treated with *Arbacia* secretion, and *Arbacia* sperm with that from *Asterias* eggs. As might be expected the phenomenon is more marked with the *Asterias* sperm on account of their original inactivity, although the *Arbacia* sperm are also noticeably accelerated. I have made no experiments capable of deciding whether the activation of the two kinds of spermatozoa is due to the existence in the secretions from the two kinds of eggs of the same substance or of specifically different substances.

3. *Re-Activation.*—Activation is a temporary state, and after certain other reactions have occurred, the spermatozoa are found to be quite immotile. Such spermatozoa, although chemically different (13^1) from fresh ones which have never been subjected to the secretions, are nevertheless capable of re-activation. This is shown by the following experiments, in which *Arbacia* sperm-suspensions prepared in each case from a single male were divided into lots, activated by the addition of secretion, observed at intervals, and treated with more secretion as well as fresh eggs. The degrees of activation, reactivation, or movement are given as great, moderate, slight, or zero. In the instances in which eggs were added, fertilizations always took place, but the proportion of eggs that divided varied inversely with the length of exposure of the sperm to the secretion (p. 369).

B. CHEMOTAXIS.

The chemotactic effect of the egg-secretion has been studied very carefully by Lillie, and both methods and results have been described by him at length ('13^1). I have verified the most essential results on *Arbacia* and have extended them to *Asterias.* The injected-drop method as well as the distribution of sperm about groups of eggs were used as indicators. As Lillie suggests, such results do not make clear the rôle of chemotaxis in normal

ARBACIA SPERM.

Experiment 1.

Treatment.	Lot A, Activation.	Lot A_1. Activation.	Lot A_2, Activation.	Lot A_3, Activation.	Lot A_4, Activation.	Time.
Added secretion	Strong	Strong	Strong	Strong	Strong	11.05 A.M.
	Moderate	Moderate	Moderate	Moderate	Moderate	11.10
Added secretion	....	Strong	....	....	....	11.10
Added eggs....	....	....	Strong	....	....	11.10
	....	Slight	Slight	....	....	11.11
	Zero	....	....	Zero	Zero	3.30 P.M.
Added secretion	....	....	....	Slight	....	3.30
Added eggs....	....	....	....	....	Slight	3.30

Experiment 2.

Added secretion	Strong	Strong	Strong	....	....	12.10 P.M.
	Slight	Slight	Slight	....	....	1.10
Added secretion	....	Moderate	....	....	....	1.10
Added eggs....	....	....	Moderate	....	....	1.10

Experiment 3.

Added secretion	Strong	Strong	Strong	Strong	Strong	12.00 M.
	Slight	Slight	Slight	Slight	Slight	1.00 P.M.
Added secretion	....	Moderate	....	....	....	1.00
Added eggs....	....	....	Moderate	....	....	1.00
	....	....	....	Zero	Zero	5.00
Added secretion	....	....	....	Slight	....	5.00
Added eggs....	....	....	....	....	Slight	5.00

fertilization. One of the difficulties is the thigmotactic response of the sperm, as this insures their sticking to surfaces they may chance to meet. It is easy to see how under such circumstances an accumulation about eggs might take place. While not conclusive, experiments in which the surface of the eggs was very materially reduced, and the jelly mass quite obliterated by desiccation, showed that even in this case the sperm collect in great numbers about the eggs. Minute fragments of egg-powder are also centers about which the sperm aggregate in great numbers. I have not observed this when powdered glass or sand were added to the suspensions and as the surfaces in these cases although of different physico-chemical properties, must be assumed to be at least as extensive as in the egg-powder, the marked sperm aggregation about the dried egg-fragments and its absence about the other fragments is certainly in harmony with the idea that chemotaxis is a factor in bringing the sperm to the egg.

C. AGGLUTINATION.

1. *Iso-agglutination.*—That egg-secretion is capable of agglutinating sperm has been fully described by Lillie ('12[1], '13[1], '13[2]). In this section I simply wish to add my testimony to his as to the facts in the case—namely that this phenomenon occurs; that it is reversible; that its duration is brief and may be utilized as a measure of concentration; and that sperm once agglutinated, although capable of re-activation, do not agglutinate again.

2. *Hetero-agglutination.*—I have found that the *Arbacia* secretion also agglutinates the sperm of *Asterias*, and that *Asterias* secretion, besides agglutinating its own, has a similar effect on the spermatozoa of *Arbacia*. Thus

1. *Arbacia* sperm + *Arbacia* secretion = agglutination masses described by Lillie.
2. *Arbacia* sperm + *Asterias* secretion = dense, angular masses. Reaction slower than 1.
3. *Asterias* sperm + *Asterias* secretion = masses smaller than 1. Angular.
4. *Asterias* sperm + *Arbacia* secretion = result similar to 3.

At the time when these experiments were performed, the material had practically disappeared, and in consequence the question whether the above hetero-agglutinations are due to the same substances as the iso-agglutinations, or whether each secretion contains both an iso- as well as a hetero-agglutinin, could not be decided. Since hetero-agglutination between *Arbacia* and *Nereis* is not brought about by the same substance ('13[1]) that causes the iso-agglutination, the same relations may hold for *Arbacia* and *Asterias*. It will prove interesting to see whether the agglutinin in *Arbacia* secretion that reacts with the *Nereis* sperm is the same one that agglutinates *Asterias* spermatozoa. If not, it becomes important to discover how many agglutinins are present. In this connection I may refer to an observation already published (13[3]), namely that the *Arbacia* secretion agglutinates the larvæ of *Arenicola*.

3. *Origin of the Agglutinin.*—Lillie has shown that the agglutinin is chiefly located in the outer jelly of the *Arbacia* egg. I have found the same thing to hold true of the *Asterias* ovum. It was also proved by Lillie (13[2]) that if the outer jelly is removed

by shaking, the eggs after two or three washings impart only a weak agglutinating power to the supernatant water. This however increases in the course of time. From this Lillie concludes that the eggs produce the agglutinating substance. Moreover he has shown that they are the only tissue of *Arbacia* that does produce this material.

My own experiments show that the agglutinating substance is located in greatest abundance in the jelly and that the eggs also contain this material. As additional evidence it may be stated that when eggs are inseminated with fairly concentrated sperm-suspensions, the collection and activity of the spermatozoa may be great enough to tear the outer jelly away from the egg. When this occurs, one may suddenly observe great balls of sperm apparently cast off from the eggs and forming huge agglutination masses. In this instance many sperm also remain in contact with the egg, which later shows a typical fertilization membrane, and divides. This point is important in connection with the mistaken idea that the outer jelly is essential for the appearance of the fertilization membrane ('13^4).

In connection with the existence of agglutinin in the egg, I may refer to experiments with egg-powder in which very effective agglutinations were secured. It cannot well be supposed that every fragment of egg-powder has bits of dried jelly adhering to it.

4. *The Mechanism of Agglutination.*—On this point Lillie's inference based on the *Nereis* sperm, in which because of relatively great size and slow movement, direct observation is possible, can be substantiated by a variety of observations. Lillie noticed that the agglutination is between the heads, and that the tails, at least until the period of paralysis sets in, are not visibly affected. "The adhesion of the heads demonstrates some change in the membrane that renders them sticky" ('13^1, p. 556), and direct observation showed "that in agglutinated masses the heads of many of the spermatozoa are swollen into spherical form and have lost the normal strong refringibility. The change is in this case a very characteristic one indicating a great change in permeability" (*loc. cit.*).

The capacity for influencing the permeability of cells is by

C. AGGLUTINATION.

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no means limited to the sperm. Thus, as I have pointed out ('13[3]), egg-extract added to blastulæ which have developed in normal sea-water, slows their movements, and increases their volume, indicating a change in permeability. R. S. Lillie has emphasized on numerous occasions the great advantages of *Arenicola* embryos ('13[5] and earlier papers) as indicators of permeability changes which may be registered by the outflow of pigment. When the *Arbacia* secretion is added to the *Arenicola* larvæ, movement is slowed down, the pigment flows freely into the water, indicating an increase in permeability, and a slight and reversible agglutination occurs. Considering all the facts at present available, it seems reasonable to suppose that agglutination is the result of an increase in permeability, and we may imagine that the exudation of material from the cells or the changes that lead to the exudation, render them sticky. Unless some other chemical reaction is involved, it seems to me more likely that the occurrence of agglutination depends upon the exudate. The sperm of *Nereis*, the blastula of *Arbacia*, and the larvæ of *Arenicola* appear to furnish us with three out of four theoretically possible types of cases.

D. PARALYSIS.

The addition of egg-secretion to a sperm-suspension is followed by activation, a chemotactic effect, and a reversible agglutination. For some time after the agglutination masses have disappeared, the sperm remain quite active, but the rate of their movements decreases until finally they come to a standstill, and appear as though paralyzed. As the re-activations show, this paralysis is not an irreversible state, although the second period of activity never lasts as long as the first, nor is the activity on the whole as great while it lasts. The third activation may be almost momentary. Re-activated sperm are capable of fertilizing the eggs.

II. QUALITATIVE CHEMICAL ANALYSIS OF THE EGG-SECRETION.

Although the observations recorded are important for an understanding of the nature of the egg-secretion, they have been reported at this time and in this connection chiefly for the interest they may have in relation to the factual basis of Lillie's theory of fertilization ('13^2). Analyses by other methods were attempted.

It is altogether likely, and in the case of the *Nereis-Arbacia* hetero-agglutination, definitely proved, that the egg-secretion contains more than one substance. The problem therefore presents itself of isolating these bodies. A first step toward orientation has been made by means of certain qualitative chemical tests.

The method of securing egg-secretion finally adopted was suggested to me personally by Dr. F. R. Lillie, and consists in adding to a certain number of "dry" eggs, double their volume of sea-water, and with occasional slight agitation, allowing ten minutes to elapse. At the end of this time the ova were precipitated by 100 revolutions of the centrifuge, and the supernatant fluid, a clear, golden liquid in the case of *Arbacia*, or whitish and opalescent in the case of *Asterias*, was usually carefully decanted without filtration through paper. Such solutions I have adopted as standard. With the *Arbacia* secretion the following tests were made:

1. The solution is gold-yellow in color and clear.

2. The solution is neutral to litmus.

3. Upon cooling to 0° no change was noticeable.

4. Upon boiling the color becomes faintly purple.

5. The purple coloring matter may be removed at least in part if white of egg is allowed to coagulate in the boiling solution.

6. No acid-insoluble precipitate is formed upon the addition of *n*/10 NaOH.

7. 1 or 2 drops conc. HCl produced faint cloudiness which became more distinct on standing.

8. The addition of alcohol produced no visible change.

9. Millon gave a white precipitate with no color change on boiling.

10. The biuret test was negative.

11. HNO_3 gave no ring but a faint cloudiness.

12. The xanthoproteic test gave no precipitate, but the solution turned distinctly yellow.

13. The Adamkiewicz test was negative.

14. Fehling gave no reduction.

15. Bi-subnitrate gave no reduction.

So far as these tests go, and they were repeated several times, it seems likely that reducing substances are absent, and that if proteins are present, their concentration is too low to give the ordinary reactions in their usual form. The opalescence observed upon the addition of acids, and the yellow color gotten in the xanthoproteic test, indicate possibly minute traces of protein but these may come from traces of the egg jelly.

With all the tests that made such experiments possible, the secretion was afterwards tested as to its agglutinating property, and was found in every case to still possess this power, tests 3, 4, and 5. No exact quantitative comparison as to the agglutinating strength before and after boiling was made. Lillie states ('13[1], p. 557) that the agglutinin when boiled and then allowed to stand at 95° for 30 minutes is destroyed in large part, and almost entirely if kept at this temperature for 66 minutes. In some of my experiments the secretion was brought to the boiling point, in others it was boiled 5 minutes. The color change noted always occurred, but the agglutination power appeared undiminished. Both of these results are described in Lillie's paper ('13[1]).

As to the nature of the purple substance, I may say that even if its formation, and presumably with that, the abstraction of something from the original solution makes no measurable difference in the agglutinating power of the secretion, this material may nevertheless be significant in other connections. My reasons for suggesting this are that when a sperm suspension is added to the secretion, traces of this purple color appear; when dilute sperm suspensions are killed by heat the same color is seen; in concentrated suspensions the red dominates over the blue; and in still more concentrated suspensions the color is like that of port wine. This same color also appears in desiccated eggs as well as sperm. From these facts the thought lies near at hand, that we are here dealing with the production of a compound specific for *Arbacia*. Corresponding experiments with *Asterias* do not give this color, nor have I gotten it with oyster sperm. On the other hand, *Asterias* sperm as well as egg-secretion turn a slight salmon-color when boiled. With the exception of these color differences, the same results in tests, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, and 14, were gotten with the *Asterias* secretion.

III. THE SECRETION OF SUBSTANCES AT FERTILIZATION AND IN HYPERTONIC SEA-WATER.

The unfertilized eggs of *Arbacia* show no noticeable decrease in volume in sea-water even four hours after removal from the ovaries ('14[1]). Fertilized eggs however are measurably smaller in both *Arbacia* and *Asterias* within a few minutes after insemination ('13[2], '14[1]). A change in the same sense seems also to occur in the lamprey ('14[2]).

The decreased volume is probably chiefly due to the loss of water but it can be shown that in *Arbacia* other substances also leave the egg.[1]

The first thing that occurs to one in testing whether at fertilization more soluble substances are secreted than from unfertilized eggs, is to compare the agglutination strength of equal quantities of sea water, with and without sperm, to which equal quantities of unfertilized eggs have been added and allowed to remain for equal lengths of time. Such experiments show that there is more free agglutinin present in the sterile water. The reason for this is not necessarily that the eggs at fertilization secrete less of this substance, for Lillie has shown that the sperm binds the agglutinin, a fact also indicated by the experiments on re-activation without re-agglutination. Moreover it is quite possible that at fertilization other substances are secreted and combine with the agglutinin outside the egg. This alone or in conjunction with the circumstance that a portion of the agglutinin in these experiments would already be bound by the sperm in the infected sea-water would account for the finding of a smaller amount of free agglutinin by any subsequent agglutination test.

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[1] McClendon ('09) says of *Arbacia:* "When the egg is fertilized or put in 'membrane-forming' solutions a fluid is extruded which pushes the jelly out from the surface of the egg." As neither the evidence for this statement, nor any reference to the fact that something is also extruded from the unfertilized eggs, are given, I do not know whether my contention is antedated or not. McClendon also states that parthenogenetic reagents, when sufficiently concentrated cause the diffusion of pigment from the eggs into the sea-water. If these concentrations are identical with those most favorable for the initiation of cleavage, there is here an important point of identity between the artificial and normal induction of development.

Such experiments therefore are not capable of deciding whether more or less soluble substance is secreted at fertilization than before. That no agglutinin is secreted after fertilization is complete, has been demonstrated by Lillie's ('13[2]) later work.

The second possibility, that of utilizing the xanthoproteic test suggested itself, for in case the yellow color were not due to traces of dissolved jelly, it might increase as the result of fertilization. As a matter of fact the yellow color gotten from sea-water over eggs inseminated as above is deeper than that given by an equal quantity of sterile water exposed to an equal quantity of unfertilized eggs for the same length of time. This was considered a promising result until it was found that the addition of even a small quantity of sperm increases the density of the color to a marked degree.

A third test however gave comparative results which are not open to this difficulty. In these experiments I made use of the fact that the unfertilized eggs of *Arbacia* discolor the sea-water. Three sets of observations were made. In each of these 1 c.c. of an egg-suspension of given concentration in which the ova were uniformly distributed, was added to each of three tubes, one of which contained 5 c.c. of sterile sea-water, the second 5 c.c. of sea-water infected with just enough spermatozoa to be very faintly opalescent, whereas the third contained 5 c.c. of "double" sea-water, *i. e.*, sea-water which had been boiled down to half its original volume. Color tests were made in the usual way, and the discoloration produced by the unfertilized eggs in a given time was taken as unity. On this basis the following results were obtained in an experiment exactly representative of the others:

	A	*B*	*C*
Time.	5 C.c. Sea-water, 1 C.c. Eggs.	5 C.c. Infected Sea-water, 1 C.c. Eggs.	5 C.c. Double Sea-water, 1 C.c. Eggs.
9.40	Color = 0	Color = 0	Color = 0
9.50	Color = 1	Color = 1.5	Color = 2
9.55	Color = 1	Color = 1.5	Color = 2
9.56	+ 1 c.c. eggs	+ 1 c.c. eggs	+ 1 c.c. eggs
10.10	Color = 2	Color = 3.0	Color = 4

The extra c.c. of eggs was added at 9.56 to see whether the first difference between *A* and *B* could be the result of either the slight opalescence due to the presence of the small amount of sperm in *B*, or to the small traces of the purple "sperm-agglutinin" compound which was necessarily formed in this tube. However the relative densities of the colors in the three tubes did not change, although the relative effect of the sperm as well as of the purple compound could not have been as great at 10.10 as at 9.50, and 9.55. The same experiment was repeated several times with more dilute suspensions of sperm, and very dense suspensions of eggs. Exactly the same relative discolorations were obtained. I mixed up the tubes on several occasions and asked some one not familiar with the experiment to see if any differences could be noted. I also had the tubes mixed and handed to me for identification. In every case it took but a moment to distinguish the tube in which fertilization had occurred. This was true also in the absence of the tube containing the double sea-water.

One may say therefore that when equal quantities of unfertilized *Arbacia* eggs are allowed to stand for equal lengths of time in equal quantities of sterile and sperm-infected sea-water, the discoloration of the supernatant liquid is greater in the case of the eggs undergoing fertilization. This proves that something in addition to water leaves the eggs at fertilization, a circumstance not at all surprising in view of the fact that, whatever else it may involve, fertilization is accompanied by an increase in permeability.[1] The question whether there is in addition to the quantitative difference, also a qualitative one, must for the present remain open.

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[1] Loeb ('13^{6}) criticizes McClendon's evidence for increased permeability after fertilization on the ground that more than one interpretation of the experimental evidence is possible. My contention is not that the permeability of fertilized eggs is greater than that of unfertilized, but that there is an increase in the permeability of unfertilized eggs at the moment at which they are being fertilized. This idea is expressed by R. S. Lillie, p. 290, "The Physiology of Cell Division," III. Direct observational support for this view is furnished by F. R. Lillie's work on *Nereis*, and more indirect evidence by my measurements of the rate of secretion by unfertilized eggs as compared with eggs undergoing the process of fertilization. The decreased volume of the *Arbacia* and *Asterias* ovum after fertilization seems to me unintelligible except as the result of an increase in permeability. Why it should be assumed that this increase is more than momentary, I fail to see.

IV. ANALYSIS BY MEANS OF THE RATE OF CLEAVAGE.

A. The Effect of Egg-Secretion in Normal and Alkaline Sea-Water.

A first step toward an answer however has been taken in the form of experiments in which known quantities of egg-secretion were added to normally fertilized eggs and the rate of cleavage compared with controls. The secretions used in these experiments were standard, prepared as before.

Many notes were taken in making these comparisons for it could not be foreseen which details might be utilized as indicators of the relative rates of development. Of all these however only certain data with respect to the 2, 4, and 8-cell stages are reported. This choice depends entirely on the advantage of reporting the more easily verifiable facts, and not because other details are in any way contradictory. Indeed the reverse is true and applies to such stages of the division spindles as can be clearly recognized in the living egg. Similar conclusions also can be drawn from the later stages, although it is much more difficult to tell at a glance whether a certain culture of blastulæ, gastrulæ or plutei, is more or less advanced than a given standard. I wish to emphasize the fact however that these later stages were obtained in large yields in the experimental cultures and were normal, though often somewhat slower of movement.

Several ways of comparing the rates of cleavage are open. Of these the following two were adopted: the time (minimum, in the table) that elapsed between insemination and the first 2, 4, or 8-cell stage seen in a particular culture is given, as well as the interval (maximum, in the table), between insemination and the time at which the cultures were at the height of these respective stages. The results of the second way of counting are in the same sense as the others, but are less accurate since there is greater opportunity for errors of judgment, whereas no error of judgment is likely with respect to the recognition of the first 2, 4, or 8-cell stage seen. The possibility that the particular cases observed were not the first to appear in the cultures of course remains, but a moment's examination of Table I will show that with very considerable errors of this sort consistent results could hardly have been obtained.

The observations were so made that intervals of one minute elapsed between the readings. Thus if *A*, *B*, *C*, *D* represent four culture dishes, *B* was read one minute after *A*, *C*, one minute after *B*, *D* one minute after *C*, and the second reading of *A* was taken four minutes after the first. Under such conditions one could introduce quite a large error from the mere arrangement of the dishes. This was guarded against both by reversing the order of reading from time to time, as well as by changing the order of the dishes in the several experiments. As a matter of fact the significant differences in my tables are always greater than the maximum error which might have resulted from the order of observation.

In the presentation of the results I have not compared the intervals for each of the three stages considered, separately (although the data for such comparison are given), but the average of the intervals that elapsed between insemination and the 2, 4, and 8-cell stages respectively. This procedure further eliminates errors attaching to any specific observation, besides reducing the number of comparisons necessary.

In the right hand division of the following table are contained comparable observations made in sea-water whose C_{OH} had been raised by the addition of 1.75 c.c. *N*/10 NaOH to 100 c.c. sea water. These experiments are included because they serve as checks on those in normal sea water. The theoretical considerations which prompted them were based on the fact brought forward by Loeb ('98) that the development of *Arbacia* is accelerated in alkaline sea-water, and depressed in acid. This is easily verified if normally fertilized eggs are allowed to develop to the blastula stage and are then divided into three lots, one for control, a second to which NaOH is added in the proportion of 1.75 c.c. *N*/10 per 100 c.c. of sea water, whereas the third is acidulated with HCl in the proportion of 1.75 c.c. *N*/10 per 100 c.c. of sea water. In such an experiment gastrulæ, with only here and there a short armed pluteus, predominate in the HCl culture at a given hour; the control at the same time contains a large number of plutei in various stages and some late gastrulæ, whereas the NaOH dish holds practically nothing but complete plutei. Loeb, in the paper referred to attributed this result to the probable acceleration of the oxidations due to the inc in OH ions. I used the NaOH in conjunction with the secretion on the theory that if the secretion affects the oxida it should antagonize the effect of the NaOH, since the secr alone, in the concentrations employed, retards the developr As measured by the first three cleavages however, the e are not antagonistic, but additive, for the NaOH if anythin an effect exactly the reverse of the expected and that act found for the later stages. This result is in harmony with L later work. In his recent book ('13[6]), p. 35, he says: "The w published years ago a paper in which he showed that the dev ment of the eggs of *Arbacia* is retarded and finally inhibit increasing quantities of acid are added to the sea water. H since vainly attempted to show that the rate of developme the sea urchin egg can be increased with the increase of the centration of hydroxylions in the sea water." If the refe here is to the paper cited above, evidence is there given tha increase in hydroyxlions does accelerate the development o *bacia*, but this acceleration was not noticed clearly until the after fertilization. Both results seem really to be correct, the rate of cleavage is either not accelerated or even depre whereas the rate of development from the blastula to the plu is accelerated.

TABLE I.

RATES OF CLEAVAGE IN MINUTES AFTER INSEMINATION.

Cells.	Sea-Water 2 Vols.		Sea-Water 1 Vol. Secretion 1 Vol.		NaOH Sea-Water 2 Vols.		NaOH Sea-Water 1 Vol. Secretion, 1 Vol.		
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	
2	44′	53′	47′	59′	45′	52′	54′	77′	Experiment
4	64	74	73	92	52	74	72	93	
8	83	91	.....	.....	74	95	.....	.....	
2	48	52	47	70	53	80	51	70	Experiment
4	48	71	55	83	80	.....	70	88	
8	71	84	63	88	85	.....	88	.....	
2	39	53	41	66	40	46	41	64	Experiment
4	42	74	66	91	46	76	.....	77	
8	.....	92	95	.....	82	93	94	.....	
2	45	67	50	74	42	59	61	79	Experiment
4	48	72	50	88	44	86	73	109	
8	72	107	98	113	70	98	.....	.....	
Average rate....	55	74	62	82	59	76	76		for first three divisi
Average rate....	47	65	54	78	50	66	64	82	for first two div

It is apparent from these figures that whether we base our averages on the time that elapses between insemination and the minimum number of a given stage or the maximum, the results are always in the same sense. The special calculations based on the 2 and 4-cell stages have been made for all the columns in order to make fair a comparison of the first three with the last in which the records do not include the 8-cell stage. It seems reasonable to assert that the presence of egg-secretion in certain concentrations retards the early development of the *Arbacia* ovum, and that the presence of NaOH in definite concentrations increases the retardation.

B. The Effect of Egg-Extract in Sea-Water.

Experiments with egg-extract, corresponding to those with egg-secretion were made for the purpose of determining whether the egg contains, and is therefore possibly able to secrete during the brief period of increased permeability incident to fertilization, substances whose effect on the rate of development might be different from that of the secretions heretofore used. Extracts were made by the use of hypertonic sea-water ("double sea-water"), by grinding the eggs with pulverized glass as well as by laking them in distilled water. The quantities and concentrations used corresponded as nearly as possible to the amounts of secretion employed in the earlier experiments although other concentrations were tried. In all cases marked retardation of development was observed. Owing to the fact that the extracts prepared by the three methods are not identical and that their analysis has not been carried far enough as yet, accurate comparisons with the secretion are at this time impossible. However three characteristics of extract-cultures contrasted sharply with cultures developing in the presence of secretion. These were: numerous arrests of development in the early cleavages; much cytolysis; and a very general failure to get beyond the early non-motile blastula. These results indicate a qualitative difference between the secretion and the extract.

V. THEORETICAL.

The heightened rate of oxidation, the increased rate of secretion together with the decrease in volume on impregnation, all suggest as the essential point in the initiation of development, the relief of antagonistic conditions. When I found that the egg-secretion in certain concentrations actually retards development, I thought I had located the antagonists, and, without knowledge of the earlier and similar suggestion by R. S. Lillie ('09), postulated that initiatory agents are effective "because through increased permeability of the plasma film the egg is enabled to loose substances antagonistic to oxidation" ('13[2], p. 450).

It would of course have been more conservative to say "antagonistic to development," for the retardation brought about by the secretions and extracts may be the outcome of interferences with other conditions and processes, no less essential for normal development, than the oxidations. Indeed Loeb and Wasteneys ('11) have demonstrated the independence of the temperature coefficients of oxidation and cleavage, so that retardation of the latter is not synonymous with a depression of the former. Again the secretion, in the concentrations in which it was employed, brings about abnormal permeability relations which might account for the retarded development, and the possibility that the substances involved have one effect in the concentrations in which they occur in the unfertilized egg, but different effects in the higher concentrations of the experiments, must not be overlooked for good analogies are to be found in the effects of different concentrations of ether (R. S. Lillie, '12[2], p. 373). While it may yet be true that the delaying effect of the secretion is actually the outcome of a depression in the rate of oxidation, proof of this must be sought in further experiments.

However, even if further experimentation should succeed in tracing the retarded development to a decrease in the oxidation rate brought on by the presence of the egg-exudate, a difficulty would still remain, for the surface film of the unfertilized ovum is permeable for the secretion, and so by constant elimination an accumulation of the suspected antagonist to the inhibition point would be automatically prevented. This is the argument applied by Loeb in contraversion of R. S. Lillie's idea that CO_2 might be the antagonist, for inasmuch as CO_2 "is a good agency for calling forth membrane formation" and as only substances capable of diffusing into the egg can have this effect, the egg surface must be permeable to it before impregnation, and consequently there can be no accumulation that could be relieved as the result of the increase in permeability associated with fertilization.

However two possibilities remain; either the quantitative difference in the rate of secretion between unfertilized ova and ova undergoing fertilization is significant, or there is a qualitative difference as yet undetected between the secretions from the two kinds of eggs. Since "the velocity of segmentation in eggs fertilized by two spermatozoa is identical with that found in eggs fertilized by one. . . . ," we must believe, according to Loeb ('13^6, p. 13) "that the spermatozoan causes development . . . by removing an obstacle to development."

Certain facts and considerations to be dealt with in a later paper, seem to point to the possibility that in addition to the quantitative difference, the secretion at the moment of fertilization differs from the secretion of uninseminated eggs, qualitatively as well. Should further experimentation establish the correctness of this suspicion, then since the rate of oxidation in fertilized eggs is greater than in unfertilized, we could still say, "substances antagonistic to oxidation are eliminated at fertilization" although these substances may be neither CO_2 nor any of the constituents of the secretion from unfertilized eggs. Furthermore their action may be indirect through the inhibition of processes which when set going by their removal allow oxidation to proceed at the heightened rate normal for the fertilized egg. Whatever the final solution of these problems may be, it seems altogether likely that the initiation of development, and with it, the initiation of the processes leading to cell division, are in some way significantly related to the momentary increase in the permeability of the ovum, accompanying the process of fertilization.

VI. SUMMARY.

1. In corroboration of F. R. Lillie, it was found that the egg-secretions of *Arbacia punctulata* exert a chemotactic effect on sperm, and activate, agglutinate, and paralyze them.

2. The egg secretions of *Asterias forbesii* behave in a similar manner toward *Asterias* sperm.

3. *Arbacia* secretion activates, agglutinates, and paralyzes

Asterias sperm, and *Asterias* secretion has the same effects on *Arbacia* sperm.

4. Paralyzed sperm may be reactivated but not reagglutinated.

5. The egg secretions test negatively for reducing substances and do not give the usual protein tests although they were found to be faintly positive to the acid tests and the xanthoproteic. This may be due to traces of the egg jelly.

6. Agglutination may be gotten with dry egg powder.

7. The agglutination reaction very possibly depends on a surface effect.

8. More soluble substances escape from the egg of *Arbacia* in hypertonic sea-water and in sea-water infected with sperm, than from unfertilized eggs in normal sterile sea-water in the same length of time.

9. The egg-secretion in certain concentrations retards development measurably.

10. 1.75 c.c. $N/10$ NaOH added to 100 c.c. of sea water does not accelerate the early cleavages. The retardation noted may well be within the limits of error.

11. NaOH in the same concentration does markedly accelerate the development of blastulæ into plutei.

12. Egg secretion in the concentrations employed + NaOH in the concentration given above, results in a more marked retardation of cleavage than the egg-secretion without the NaOH.

13. Egg-extract, as contrasted with egg-secretion, in addition to retarding development, in a similar manner when employed in a similar manner, results in cytolysis, arrests of development in the early cleavages, and a general failure of the eggs to get beyond the early non-motile blastula.

14. The heightened rate of oxidation in the fertilized egg; the increased rate of secretion in eggs undergoing fertilization; the decreased volume after fertilization all point toward the possibility that initiation of development depends upon the removal of substances, directly or indirectly antagonistic to oxidation. Proof that the egg-secretion in certain concentrations measurably retards development is however insufficient evidence either for the conclusion that it itself is the antagonist, or contains it.

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This would not follow even if it were shown that the retardation is the result of depressed oxidation. It is possible however that egg-secretion, at the moment of fertilization differs qualitatively from the earlier secretion, and contains the real antagonists whose inhibitory effect need not necessarily be thought of as having been direct.

15. These suggestions together with the facts upon which they are based, are not necessarily out of harmony with existing prominent theories of fertilization.

University of Michigan,
December 20, 1913.

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ON AUTO-PARTHENOGENESIS IN ARBACIA AND ASTERIAS.

OTTO Charles GLASER.

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ON AUTO-PARTHENOGENESIS IN ARBACIA AND ASTERIAS.

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The unfertilized ova of *Arbacia* eliminate substances which discolor the sea-water, but when the same eggs are undergoing impregnation, the discoloration of an equal amount of sea-water in unit time, is roughly half again as much ('14[2]). Hand in hand with this fact goes the discovery that both *Arbacia* (*punctulata*) and *Asterias* (*forbesii*) ova, are measurably smaller in volume after fertilization than before ('13[1], '14[1]). These observations led me to begin an investigation which might throw some light, not only on the nature of the substances lost, but on the further question whether their elimination is significantly associated with the initiation of development. Not more than a beginning has been made, but some of the preliminary, chiefly qualitative, results are sufficiently interesting to be reported at the present time.

My earliest experiments consisted in a series of attempts to find what sort of substances could be gotten out of the eggs. I therefore prepared solutions in sea-water from ground, laked, or extracted ova. These preparations when tested with sperm exhibited the properties which had been described by F. R. Lillie for egg-secretion ('12, '13[2,3]). Emphasis must be laid on the power of these extracts to activate the sperm; on their chemotactic effect; and on their sperm-agglutinating as well as sperm-paralyzing capacities. Addition of the extracts in certain concentrations to normally fertilized eggs, resulted in a retardation of development; normal blastulæ instantly slowed their movements, and underwent a noticeable increase in volume when subjected to the extracts. Similar observations were made on the larvæ of *Arenicola* whose rate of movement was also slowed down, to be followed instantly by an outflow of their yellow pigment and a slight and reversible agglutination.

From these preliminary observations it appeared that the egg-extracts are capable of increasing the permeability of cells. On the view therefore that an increase in permeability is associated, significantly or otherwise, with fertilization, the initiation of development by means of these extracts should be possible. Against this idea stood the evidence of Loeb ('11) according to which "it was found that it was absolutely impossible to cause membrane formation of the sea-urchin egg by extracts from the sea-urchins of the same or even related species." From this Loeb concluded that the cortical layer of the egg exhibits against the lysins of the body producing it, the immunity characteristic for genetically related cells in general. It seemed little likely therefore that extracts prepared from the cells themselves should result in the initiation of development, and as a matter of fact, "membrane formation" in the sea-urchin egg did not occur. On the other hand, in corresponding experiments on the eggs of *Asterias*, a typical fertilization membrane did appear, and in both sea urchin and starfish, development was successfully initiated. For the type of initiation here dealt with, I have chosen the term auto-parthenogenesis.

AUTO-PARTHENOGENESIS IN *Arbacia* BY MEANS OF EGG-SECRETION.

The solutions employed in these early experiments, despite the use of weighed quantities of eggs, were not only very variable in concentration, but also differed in composition according to the method of preparation ('14^2). Since the secretion from unfertilized eggs, has the same effects on sperm as the extracts, it appeared possible that it might also have similar effects on blastulæ, *Arenicola* larvæ, and unfertilized ova. This indeed was soon found to be the case, although as pointed out elsewhere ('14^2) extract and secretion are probably not identical throughout.

The next step, the discovery of a more reliable mode of procedure, resulted in a definite method of securing results, but I have no reason for considering it final. Omitting the experiments of orientation and other unnecessary details, the following outline may be taken as a guide for further work: Standard secretion was prepared by adding to a certain number of "dry" ripe ovarian eggs, double their volume of sea-water. At the end of ten minutes, during which the eggs were slightly agitated at intervals, the suspension was centrifugated, and the eggs cast down. After 100 revolutions the supernatant fluid was carefully decanted and set aside for use.

Ripe eggs were then shaken, usually from the ovaries of a single individual, into a small quantity of fresh sea-water, and to 1 c.c. of a concentrated suspension of these, was added 1 c.c. of the secretion. In this mixture the eggs were allowed to stand 2 hours, when cleavages were usually found in all the dishes. That these were not the result of accidental infection with sperm was guaranteed by special precautions—all dishes, instruments and the hands as well as the animals used having been carefully sterilized. Furthermore, when the first urchin opened was a female, I usually sought no further, as the eggs from a single individual are sufficient in number. Indeed in all but two or three series only eggs from a single urchin were used. It might be urged that the secretion or the sea-water were infected. Sterilization of the latter however does not prevent auto-parthenogenesis, and against the idea that the sperm might have been present in the secretion there are three arguments, two of which are conclusive. In the first place, as has been shown by F. R. Lillie ('13[2]) and myself ('14[2]), the fertilizing power of sperm decreases markedly if they are allowed to remain in the secretion more than a few hours. In some of my experiments the secretion was prepared a day in advance, and used on the eggs of the first urchin opened on the following morning. In the second place fertilization membranes never appeared in any of the experiments with *Arbacia* eggs unless especially induced, and lastly, if the same eggs from which the secretion was prepared were afterwards subjected to its influence for the proper length of time and in the proper concentration, characteristic auto-parthenogenesis took place.

Many experiments were tried varying the concentration of the secretion as well as the time of exposure. My records indicate cleavages at higher concentrations as well as lower, and also in less than two hours, but the greatest number was always obtained when 1 volume of the concentrated egg suspension was exposed for 2 hours to 1 volume of the standard secretion. If at the end of this time the supernatant fluid is poured off and replaced by fresh sea-water, free-swimming blastulæ will be found within 24 hours. In one case only did development proceed to the pluteus stage.

Among the blastulæ that develop in these cultures are some characterized by an extremely slow rate of movement; others, though rare, may move at a practically normal rate, whereas the majority are considerably below normal in speed. This fact in earlier experiments misled me, as I caught sight of only the fastest swimmers, and mistook the others for arrested cleavages.

The size of the blastulæ is also a ready source of deception, since there are in general three kinds, micro-blastulæ, normal-sized blastulæ, and mega-blastulæ, of which the first are by far the most numerous. As these are usually very transparent and also slow of movement their presence in a culture may easily be overlooked.

The origin of the micro-blastulæ is clear. The first two cleavage cells, although normal in size, in most cases do not remain together, but separate and proceed to develop independently of one another. If the cells produced by these isolated blastomeres continue to separate very minute micro-blastulæ may result. Such falling apart of the cleavage products has been observed in the 16-cell stage and even later. The separation takes place, I believe, because the fertilization membrane has not appeared ('13[1]).

Normal-sized blastulæ need no explanation, since they must result from the eggs whose cleavage cells can be seen to hold together despite the absence of the normal fertilization membrane. As for the mega-blastulæ, it is possible that their origin is identical with that of mega-blastulæ in normal cultures. On the other hand, they were more noticeable in the auto-parthenogenetic series, and it is possible that the larger type of egg is better suited for this than the normal mode of development. Again the possibility is not excluded that the mega-blastulæ may come from eggs of normal size. This is not unlikely, since normal sized blastulæ instantly increase in volume when subjected to the same concentrations of the secretion.

IMPROVED METHOD OF AUTO-PARTHENOGENESIS.

Loeb's improved method of artificial parthenogenesis consists in following the treatment with parthenogenetic agents, by an after-treatment with hypertonic sea-water, 8 c.c. of 2.5 *M* NaCl + 50 c.c. sea-water. It seemed likely therefore that a better yield of larvæ could be secured if eggs, after having been subjected to the action of the secretion for two hours, were afterwards treated with the hypertonic solution for forty minutes. This surmise proved correct. The following is an outline of a typical experiment:

Time.	*A*	Time.	*B*
10.20 A.M.	1 c.c. eggs + 1 c.c. secretion.	10.12 A.M.	1 c.c. eggs + 1 c.c. secretion.
12.12 P.M.	Began hypertonic treatment.	1.12 P.M.	Began hypertonic treatment.
12.52	Ended hypertonic treatment.	1.52	Ended hypertonic treatment.
2.30	Considerable number of 2, 3, and 4-cell stages.	2.45	Many cleavages; 2–8 cells. Many normal.
5.45	Numerous 2, 3, and 4-cell stages.	6.00	Many 2, 4, 32, 64 and 128-cell stages.
	Very few 32–64-cell stages.	7.30	Cleavages in every field.
9.30 A.M.	1 active larva, many dead.		Some blastulæ but not free.
		9.45 A.M.	12 fine active larvæ. 50 per cent. of cleavages arrested.
		4.00 P.M.	Hundreds of slowly moving blastulæ.

In experiment *B* the time of exposure to the secretion was three hours instead of two as in earlier experiments with the hypertonic after-treatment. From this one might infer that better results could have been obtained in the experiments in which the secretion alone was used, but the results do not warrant this assumption. Controls also show that with the hypertonic after-treatment, three hours of exposure to the secretion is better than two. We must believe therefore that the success of experiment *B* and all the others carried out in the same way, depended on the hypertonic after-treatment.

The differences in the length of exposure to the secretion are for the present purely empirical.

In all the experiments both with as well as without the hypertonic after-treatment, development was much delayed in many of the eggs, and rarely went beyond the blastula stage. As stated before, I observed plutei only once, and not over ten or twelve at that. Gastrulæ also were rare. For this failure to pass beyond the blastula stage there are several reasons; in the first place one must attribute a large share to the fragmentation of the early cleavage stages, due as I believe to the absence of a normal fertilization membrane, and secondly the abnormal permeability relations resulting from the treatment with the secretion probably also play a rôle ('14^2) by placing mechanical difficulties in the way of gastrulation even though the necessary materials are present in the embryo. The evidence for this conclusion cannot be presented now. Although these obstacles stand in the way of perfecting this method of rearing sea-urchins, the fact that it is impractical, and inferior to other methods does not in the least detract from the theoretical interest of the results. As McClendon ('09) puts it: "In natural parthenogenetic development the end result may be a maturation or segmentation stage or a larva or adult. Though only the reproductive adults are of significance to the species, all are of significance to science."

AUTO-PARTHENOGENESIS IN *Asterias*.

In earlier papers ('13^1, '14^1 and '14^2) I have emphasized certain differences as well as certain points of similarity between the eggs of *Arbacia* and *Asterias*. Chief among the differences, are the characteristics of the fertilization membranes; most important among the points of likeness are the decrease in volume to be observed in both kinds of eggs on fertilization, and the fact that the *Asterias* ovum produces a secretion in which the most striking characteristics of the corresponding *Arbacia* exudate can be verified point for point. For these reasons one might anticipate the initiation of development in the *Asterias* egg by the proper employment of its own secretion.

In the following experiments the secretion was prepared exactly as in the case of *Arbacia*, but the eggs before treatment were given time to mature, *i. e.*, they were allowed to stand for about 1 hour in sea-water after removal from the ovaries.

10.27 A.M.	1 volume *Asterias* eggs. 1 volume *Asterias* secretion.	10.27 A.M.	2 volumes *Asterias* eggs. 1 volume *Asterias* secretion.

10.50	Fertilization membranes indicated.	10.53	Fertilization membranes indicated.
12.15 P.M.	Many eggs elongated.		
2.00	Fertilization membranes. Frequent cleavages.	2.00 P.M.	Frequent cleavages.
9.00 A.M.	Cytolysis. One free swimming blastula.	9.00 A.M.	Numerous dead blastulæ. Much gastrulation.

In other tests similar results were obtained. Unfortunately this work came at the end of the *Asterias* breeding season, and further experiments were impossible. Those performed however are sufficient to show that parthenogenesis can be induced in *Asterias* as in *Arbacia* by the use of its own egg-secretion. Owing to the existence of a perivitelline space within which the cleavage products of the *Asterias* egg are held, it appears likely that better results will ultimately be achieved than in the auto-parthenogenesis of *Arbacia*. In fact as indicated above, even without hypertonic after-treatment, which in first experiments was always omitted for the sake of control, one of my auto-parthenogenetic *Asterias* cultures proceeded to the gastrula stage.

"HETERO-PARTHENOGENESIS."

"Hetero-parthenogenesis" as the sequel will show, is probably not the correct word, but I use it here as a convenient heading for experiments in which development was initiated in *Asterias* eggs by the use of *Arbacia* secretion. Such experiments suggested themselves, as the result of the earlier observations on the increase in the permeability of various types of cells. From these one might anticipate "hetero-parthenogenesis," a process which was indeed successfully induced as the following experiment indicates:

10.35 A.M.	1 volume mature *Asterias* eggs. 1 volume *Arbacia* secretion. No immediately visible effect.
10.50	Fertilization membranes indicated.
2.00 P.M.	Numerous cleavages.

THE FERTILIZIN THEORY.

After I had discovered auto-parthenogenesis through the observation that the egg-extracts are permeability increasing agents, I consulted with Professor F. R. Lillie who had been performing those experiments on normal fertilization in *Arbacia* which have led him to propose the fertilizin theory, briefly outlined in his paper "The Mechanism of Fertilization" ('13[3]).

The facts on which this theory is based are dealt with in F. R. Lillie's earlier papers, as well as in the one specifically referred to. In my paper on "A Qualitative Analysis of the Egg-Secretions and Extracts of *Arbacia* and *Asterias*" ('14[2]) will be found certain verifications of Lillie's observations. It would take us too far to go into the factual basis of the theory here, but briefly it postulates as the essential point in fertilization a chain of chemical reactions in which an amboceptor-like substance—the sperm-agglutinating agent—present in the secretions from the egg, unites by means of a spermophile side-chain with receptors in the sperm, and by an ovophile side-chain with receptors in the egg.

If this chain of reactions indeed occurs and is related to fertilization and the initiation of development in the significant manner put forward by Lillie, it follows that there are theoretically at least five possibilities of blocking fertilization. Lillie has listed these as follows:

1. Through loss of fertilizin by the egg.
2. Through occupancy of the sperm receptors.
3. Through occupancy of the egg receptors.
4. Through occupancy of the ovophile side chain of the amboceptor (fertilizin).
5. Through occupancy of the spermophile side-chain of the amboceptor (fertilizin).

Of these Lillie has proved possibility 1, by washing the eggs; 4, by means of an inhibitor in the blood which prevented fertilization, but not the sperm-agglutinin reaction; and 5, by setting free from the egg itself an inhibitor, the anti-fertilizin which obstructs the union of the sperm with the amboceptor by occupying the spermophile side-chain. Since therefore the presence of the agglutinating agent, and moreover its presence in a state in which both spermophile and ovophile side-chains are free to combine with their respective normal receptors in the sperm and the egg, seems to be necessary for normal fertilization, Lillie has called the substance in question, fertilizin.

F. R. Lillie has also offered an interpretation of my results on auto-parthenogenesis. He suggests that in the initiation of development by employment of the egg-secretion, essentially the same chemical chain is involved as in normal fertilization except that the sperm, and naturally the sperm-fertilizin reaction also, is dropped. In other words, in auto-parthenogenesis, the egg-receptors are thought of as combining with the ovophile side-chains of fertilizin which has not been bound to sperm-receptors through its spermophile groups.

Off-hand this supposition does not appear unreasonable. The fertilizin is certainly present in abnormally high concentration in the secretion as used for auto-parthenogenetic initiation, and the mere fact that the secretion is a permeability-increasing agent does not preclude the possibility that one of its constituents may play an additional rôle in the initiation of development. If this is true, it should be possible to block auto-parthenogenesis in accordance with the scheme outlined above, although the absence of the sperm and the sperm-fertilizin reaction restricts the opportunities to 1, the loss of fertilizin; 3, the occupancy of the egg-receptors; and 4, the occupancy of the ovophile side-chain of the fertilizin.

METHODS OF PREVENTING AUTO-PARTHENOGENESIS.

1. *The Prevention of Auto-parthenogenesis by Washing the Eggs.*—Several preliminary experiments in which the eggs before being exposed to the action of the secretion were washed from 20 minutes to half an hour in great excesses of sea-water, proved conclusively that this procedure very materially reduces the number of cleavages, and the number of larvæ, afterwards found in the cultures. These results led to a more careful test carried out on eggs which had been washed in excesses of sea-water for 24 hours. The sea-water was changed frequently but at irregular intervals. These eggs were then treated in the usual way with the secretion, but not a single cleavage was found. Thus the effect of even slight washing is noticeable whereas prolonged washing effectually prevents the auto-parthenogenetic cleavage. From this we might conclude that something essential for the initiation of development had been removed from the eggs.

However the failure of the fertilizin with which the eggs were treated and for which they are permeable, to offset the effects of washing, remains to be accounted for.

2. *The Prevention of Auto-parthenogenesis by Means of a Group Split from the Sperm.*—Lillie has made the apparently justifiable assumption that fertilizin bound by sperm is activated, *i. e.*, has a greater chemical affinity for the substances with which it unites in the egg than the unbound fertilizin. If this is true, it occurred to me that the use of sperm, in conjunction with the secretion might give better auto-parthenogenetic results than had been obtained by the use of the secretion alone, either with or without the hypertonic after-treatment. Naturally only dead or seriously injured sperm are available for this experiment, and with the latter one could not be sure to exclude a certain and misleading number of real fertilizations which would then be mistaken for very fine cases of parthenogenesis. Since the power of the fertilizin to produce the agglutination reaction and to play a part in normal fertilization is not destroyed by brief boiling, it seemed possible that the complementary chemical groups in the sperm might also survive boiling without being destroyed or so altered as to be incapable of taking some part at least in the reactions between egg and fertilizin.

However, instead of furthering auto-parthenogenesis, exactly the opposite effect was found, for the boiled sperm absolutely prevented the process, as shown by the following experiment:

1 volume of eggs+1 volume of secretion[1]+2 volumes boiled sperm infusion 10.40 A.M. to 2.50 P.M. Four hours later little if any effect visible; a few cell-masses were noted, but nothing that in any way suggested auto-parthenogenesis. At 2.58 P.M. the mixture was diluted with an excess of sea-water. At 6 P.M. no effect was noticeable; by 10 A.M. the next day, the eggs had undergone a granular decomposition.

Experiment repeated carefully controlled. Result identical. In the control however auto-parthenogenesis occurred and on the following morning 12 very fine larvæ were seen in addition to the usual number of delayed cleavages and microblastulæ.

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[1] Unfortunately the egg-secretion in these tests was more dilute than usual, but the effect noted is not interpretable on this basis since parthenogenesis occurs at greater dilutions. The sequel to these experiments further emphasizes the interpretation given here.

Since the fertilizin theory postulates essentially the same machinery for auto-parthenogenesis and normal fertilization it follows that initiation by living sperm should also be blocked by treating the eggs with the boiled sperm-infusion. This is actually the case:

> Eggs treated for two hours with strong boiled sperm-infusion. At the end of this period they were heavily inseminated with living sperm, but remained unfertilized. Only a few cleavages were observed later, but these were arrested at the latest in the four-cell stage. No further development occurred, but on the following day practically all the eggs exhibited fertilization membranes. Controls normal.

3. *The Prevention of Auto-parthenogenesis by Means of a Group Split from the Secretion.*—F. R. Lillie ('13[2]) finds the agglutinative power of egg-secretion unimpaired, or perhaps better, not measurably impaired, by boiling for 5 minutes, although the solution undergoes a change of color due to the formation of small quantities of a purple compound ('14[2]). As the secretion is thus visibly different in chemical composition it becomes important to ascertain whether the formation or presence of the purple compound makes any recognizable difference in the initiation of development. The following experiments indicate that the efficacy of the secretion as a parthenogenetic agent is blocked.

Standard secretion boiled. Purple compound formed. Sperm-agglutination positive.

3.20 P.M. 1 volume of boiled secretion added to 1 volume of fresh egg-suspension.

8.00 P.M. No cleavages.

8.00 A.M. Few delayed cleavages, not at all in normal frequency.

11.30 A.M. 1 volume of boiled secretion added to 1 volume of fresh egg-suspension.

2.00 P. M. No cleavages.

7.00 P.M. No cleavages.

8.30 A.M. No cleavages.

Control in unboiled secretion exhibited auto-parthenogenesis in usual quantity.

INTERPRETATION OF EXPERIMENTS ON PREVENTION OF AUTO-PARTHENOGENESIS.

Analysis of the means by which a process can be prevented may be even more suggestive than a similar analysis of the means by which it can be induced, and this proves to be true in the present case. How then are these experiments to be understood?

1. *The Block in Washed Eggs.*—The prevention of fertilization by washing (F. R. Lillie, '13[3]), according to the fertilizin theory, is due to the absence of the intermediary body with which both sperm and egg receptors normally unite. We cannot, however, despite the superficial similarity of the two cases, attribute the auto-parthenogenetic block to the absence of suitable bonds for the egg receptors since the surface film of the ovum is perfectly permeable for fertilizin. This must diffuse into the eggs from which it has been removed the moment they are exposed to the concentrations employed for initiation. If this is correct, then the prevention of auto-parthenogenesis in washed eggs must be explained in some other way. This however is not necessarily fatal to the fertilizin theory, for these same experiments strongly suggest that a mere increase in permeability is insufficient to initiate development, a suggestion still further reinforced by the second type of block to be considered presently.

The experiments with washed eggs are also suggestive from another standpoint. Lillie (*loc. cit.*) tells us: "If it be true that the egg contains its own fertilizing substance it might also be possible to induce parthenogenesis by increasing the concentration of this substance to a certain point. . . ." The washed eggs however demonstrate that the fertilizin experimentally added to the solution about the eggs has no fertilizing effect, and if it has none when the eggs have been washed, there is no good ground for considering its effect different when the eggs have not been washed. "Hetero-parthenogenesis" strongly backs up this position. It appears most likely therefore that in auto-parthenogenesis the fertilizin experimentally added, plays the same rôle as any other permeability increasing agent effective in the artificial initiation of development. If this is correct, and furthermore, if the parthenogenetic block in washed eggs is not identical with the fertilization block brought about by the same means, it follows, not that the fertilizin theory is inapplicable to these cases, but only that it does not apply exactly as F. R. Lillie has supposed.

2. *The Block by Means of Groups Split from the Sperm and the Egg-Secretion.*—The prevention of both fertilization and auto-parthenogenesis in eggs previously treated with boiled sperm infusion, as well as the parthenogenetic block in eggs subjected to the action of boiled secretion, seem at first sight to have little in common. In reality however there is an element of identity in all three cases, for suspensions of sperm as well as the egg-secretion give on boiling a characteristic color reaction due to the formation of a purple substance which may be looked upon as a chemical cleavage product split off from a mother substance. In an earlier paper ('14[2]) I suggested on other grounds, and in complete ignorance of its rôle in parthenogenesis, that this purple material might prove to be significant, as it seems to be specific for *Arbacia*. This suggestion now appears almost certainly correct, and I therefore propose for this substance, provisionally at least, the name "Purple X."

An attempt to understand the part played by Purple X in these reactions must begin with the proof that this substance and not some other cleavage product is really the effective one. Such proof would be found if the block to both parthenogenesis and fertilization could be removed with the removal of the Purple X. To accomplish this and at the same time not render the solutions toxic by the addition of precipitating agents, called for methods which had first to be discovered.

The Purple X is either in a state of very fine suspension, bordering on solution, or is actually in solution. Since now a precipitable colloid frequently carries other bodies with it when thrown down, I added the albumen of hen's egg to the *Arbacia* egg-secretion, and boiled the mixture. The expectation that the purple compound might be removed at least to a considerable extent, proved correct, as the white coagulum of hen's egg albumen was quite markedly discolored by the purple substance. After filtration the secretion was tested for its parthenogenetic effect. The method of separation however is not quantitative and so the results were not perfectly clear cut. Nevertheless it remains true that the only boiled secretion that ever induced parthenogenesis was one from which the Purple X had been removed by coagulating white of hen's egg. This result, I may add parenthetically was obtained only after a very remarkable effect of the egg-white itself had been investigated. Even in the dilutions in which it is present in the sea-water after boiling, the white of a hen's egg produces very regular and curious distortions of the eggs which may even be divided into pseudo-cleavage products. However the effect does not seem to be analogous to parthenogenesis, and I shall therefore deal with it separately at another time.

In the course of these experiments a very much better and simpler method was found, for the Purple X is a relatively unstable compound and readily disappears. Thus a concentrated sperm-suspension boiled on one day and giving a color as deep as port wine, may on the next be golden yellow. With such a solution eggs were treated for one hour and five minutes. At the end of that time the addition of living sperm resulted in the fertilization of practically every egg. The experiment was repeated, the eggs being exposed for two hours and forty minutes, and again fertilization occurred.

As a counterpart to these experiments with sperm, the parthenogenetic effect of boiled secretion which had lost its purple color was tried. Such solutions contrast sharply with the newly boiled secretion, for whereas the latter very effectually blocks auto-parthenogenesis, development can be induced without difficulty after the spontaneous disappearance of the Purple X.

Since the Purple X-block occurs in the presence as well as in the absence of sperm, we can be certain that it is not the result of an effect upon the male sex cells or, in the language of the fertilizin theory, of occupancy of the sperm receptors. Which particular one of the remaining three blocks theoretically possible we are dealing with however must for the present remain an open question, although if the immediate effect of the egg-secretion is really identical with the immediate effect of any other parthenogenetic agent, occupancy of the egg-receptors is indicated. If this is true, then Purple X should block parthenogenesis no matter how induced. That this process should be blocked by less than is required to prevent fertilization is perfectly intelligible on Lillie's assumption that sperm-bound fertilizin is activated and has a greater affinity for the egg-receptors than free fertilizin.[1]

AN ATTEMPT TO CONSTRUCT A PROVISIONAL WORKING HYPOTHESIS.

Although no final interpretation of the phenomena of fertilization is possible at the present time, an attempt to formulate some viewpoint which does no violence to our knowledge, and which at the same time may serve as a working hypothesis, must be made for further guidance.

If we accept the evidence of F. R. Lillie, together with my verifications and additions to it, and remember that many of the facts discovered by Lillie, as well as some found by myself, are the direct products of the fertilizin theory considered as a tool, I think we may even now admit that it is a useful working hypothesis. Lillie ('13) himself has emphasized this point, and moreover has listed among the other advantages of his theory, that it gives us an explanation of the specificities of fertilization; may furnish the foundations for the chemical conceptions needed by any theory of fertilization, and above all, that it offers one explanation for the initiation of development, whether by fertilization or parthenogenesis.

To what extent these claims are justified, and to what extent the theory will undergo modification, it would be unprofitable to discuss at present. One claim and its consequences however does invite discussion at the present time, and in this connection, for if the fertilizin theory is really capable of unifying the various methods of inducing parthenogenesis, its relations to another view, which apparently does the same thing must be analyzed, and furthermore we must answer the question whether all cases of parthenogenesis are really instances of auto-parthenogenesis.

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[1] Since Purple X also appears when spermatozoa come into contact with the secretion, it is possible that its appearance plays a rôle in the prevention of polyspermy. This would not prevent a similar effect on the part of the anti-fertilizin, as suggested by F. R. Lillie. Indeed the chances that any particular sperm will complete the reactions postulated by the theory, must be exceedingly small when we reflect on the immense numbers which collect about each egg. Such collections become more intelligible when we recall that whereas the biparental effect can be carried out by a single sperm, the initiatory effect, at least in *Arbacia* ('13[1]) requires more than five.

1. *The Surface-Alteration Theory.*—The view which we may call the surface-alteration theory is a product of the work of Loeb, R. S. Lillie, and many others. According to Loeb the initiation of development depends in the first place upon a superficial cytolysis, or destruction of the cortical layer of the egg, brought about either by a lysin in the sperm, or by the so-called parthenogenetic methods and agents. After this alteration of the surface the rate of oxidation in the egg is raised from four to six times. Loeb says:

"There are two possibilities by which this result can be produced: either a catalyzer (an oxidase) is carried into the egg by the spermatozoon; or the change in the surface layer itself causes the increased rate of oxidation. Everything speaks in favor of the second assumption."[1] Further on Loeb suggests "that the spermatozoon causes development . . . by removing an obstacle to development."[2]

R. S. Lillie ('11 and earlier papers) has suggested that the superficial cytolysis increases the permeability of the egg, and further that through this increase in permeability a substance antagonistic to oxidation is eliminated from the egg. This substance he imagined might be CO_2 ('09).

Against this idea Loeb has raised objections. Inasmuch as CO_2 "is a good agency for calling forth membrane formation" and as only substances capable of diffusing into the egg can have this effect, the egg surface must be permeable to CO_2 before fertilization, and consequently there can be no accumulation that could be relieved as the result of an increase in permeability.

This criticism seems valid, and while it constitutes a reason for giving up the idea that the antagonist lost is CO_2, it in no way bears on the other idea, namely that there is an increase in the permeability of the ovum as the result of fertilization. However this idea also has failed of acceptance by Loeb, but this is due chiefly I believe because the evidence in support of it has been unfortunate not only in a purely technical sense which has been justly criticized, but also because it was gathered under a misapprehension apparently as to exactly what R. S. Lillie had suggested. So far as I can see, experiments in which the permeability of fertilized eggs is compared with that of unfertilized have no bearing on the question, for no such difference was postulated, but only that the "increased permeability is . . . temporary in normal or in favorable parthenogenetic fertilization" ('11, p. 307).

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[1] "Artificial Parthenogenesis and Fertilization," 1913, p. 13.

[2] The obstacle "removed" is a cortical layer unsuited for development, and the "removal" is in reality an alteration of this cortex: "Through cytolysis of the cortical layer of the egg the oxidations in the unfertilized egg are accelerated from four to six times their normal rate" (loc. cit., p. 14). Since this heightened oxidation persists after fertilization, and since the permeability of fertilized eggs may not be different from that of unfertilized, it is possible that the cytolysis of the cortical layer alone is insufficient to account for the increase in the rate of oxidation.

If this idea is correct we might expect two things to happen at fertilization in the egg of *Arbacia*. Since this egg before impregnation actively secretes materials that discolor the sea-water, the discoloration produced by eggs undergoing fertilization might be greater. I have found ('14[2]) that it is one and a half times that of eggs not undergoing impregnation. Further, fertilized eggs, after the process is complete, do not discolor the sea-water. In the second place even a temporary increase in permeability should result in a change in volume if the eggs remain in a medium that remains constant during the period under discussion. Measurements show ('14[1]) that the eggs of both *Arbacia* and *Asterias* are smaller in volume after fertilization than before.

I therefore consider the idea of increased permeability *during* fertilization correct, and that we may suppose all cases of fertilization, whether with sperm or without to have this one point in common. Since many of the results now published had not been found at the time I wrote my preliminary paper ('13[1]) I threw emphasis on this common attribute in section VIII. This emphasis I do not wish to withdraw as the result of later work, for this has only strengthened my conviction that in fertilization an increase in the permeability of the ovum actually occurs. The only question at issue seems to me to be the significance of this change for the initiation of development.

On this particular point I also postulated the loss of substances antagonistic to oxidation. This suggestion was based on the fact that substances are lost during the act of fertilization at a higher rate than before, and finally that the presence of egg-secretion in certain concentrations retards development. The retardation I supposed to be due to the depression in the rate of oxidation. This assumption, as I pointed out, does not seem unreasonable; on the other hand as I pointed out more recently, retardation of development may in this case also be due to interference with, or depression of, other processes no less essential. Only further work can decide these questions.

2. *The Fertilizin Theory.*—In the fertilizin theory, no particular rôle is assigned to the increased permeability. "The nature of the effect of the activated fertilizin on the egg is analogous in some respects to a superficial cytolysis, in this respect agreeing with Loeb's theory. But the 'lysin'[1] is contained in the egg, not in the sperm as Loeb thought; if cytolysis is involved, it is a case of auto-cytolysis. This may involve increase of permeability, the effects of which R. S. Lillie especially studied" ('13[3]). These possibilities were mentioned "in order to point out that the conception" of the fertilizin theory "is not in conflict with the well-established work of others." This is very important for the fertilizin hypothesis is based on certain facts which seem to permit the interpretation given them, and the surface-alteration theory also has a verifiable factual basis. These facts, occurrences in and about fertilization, cannot be in conflict with one another. The problem is to understand their interrelations. The experiments on auto-parthenogenesis carry us a step forward in this direction.

At first sight success in initiating the development of *Asterias* eggs by means of *Arbacia* egg-secretion would appear to seal the death-warrant of Lillie's fertilizin theory. On the other hand a little reflection will show that no radical conclusions need be drawn. Parthenogenesis, as is well known can be induced in different eggs by the most diverse means—electricity, heat, cold, mechanical shock, osmotic pressure,[2] specific chemical alteration of the surface film or cortical layer, lipoid solvents, and even the prick of a needle having been found effective. These methods, each of which is capable of variation and modification, have nothing in common except their result, and it is idle to try to imagine any direct causal connection between them and the result. However one effect, common to all, seems to stand intermediate between the parthenogenetic result, and the experimental means used to induce it. This common intermediary is a change in the permeability of the surface of the eggs, and in the experiment in which parthenogenesis is induced in one ovum by the secretion of another it is not necessary to assume that anything occurs that does not also take place in all the other cases. The same reasoning applies also to auto-parthenogenesis, a process which was found by application of the surface-alteration theory, and in total ignorance of the fertilizin hypothesis.

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[1] It would have been more correct to say: it *was* contained in the egg.

[2] In the case of the purely osmotic methods, Loeb supposes that "the hypertonic solution acts simultaneously in two capacities: first as a cytolytic agent causing a change in the cortical layer (the formation of a gelatinous film) and second, as a corrective agency" ('13[4], p. 159).

The cytolytic action of the hypertonic solution is not without evidence, and so we may consider this method as having the effect common to all the others. If however the increase in permeability is effective also on account of the destruction of the normal fertilization block, in the sense outlined in the text, loss of water by the egg would directly or indirectly accelerate the loss of the antagonists (cp. Glaser, '13, p. 450).

Now the picture of fertilization with sperm given by the fertilizin theory is briefly the sperm-receptor-amboceptor-egg-receptor reaction. In the picture of auto-parthenogenesis, the sperm is dropped, leaving the amboceptor-egg-receptor to react, the former with an unsatisfied bond. Since however parthenogenesis can be induced without the addition of fertilizin to the eggs there is no reason for supposing that the egg-receptors united with the fertilizin which was added in my experiments for the purposes of initiation. This however does not seem to me to be at all necessary, for both substances are present in the egg from the beginning and we may postulate a union between the receptors and the fertilizin within the egg.

If this is indeed the mechanism of both parthenogenesis and auto-parthenogenesis, we must answer the question why the egg was not fertilized before, or what amounts to the same thing, why fertilization occurs at all.

In speaking of parthenogenetic agents, Lillie (*loc. cit.*) tells us, they "need only remove obstacles to the union of the amboceptor and the egg-receptor" (p. 527); and in connection with auto-parthenogenesis, that this might result "if the concentration of fertilizin were raised to a certain point, though it is conceivable that no increase in concentration would break down the resistance that normally exists to the union of the amboceptors and egg-receptors" (527). In other words, the egg is not always fertilized, because there are obstacles or resistances. When these are removed, fertilization, parthenogenesis, or auto-parthenogenesis may occur.

No circumstance connected with the initiation of development that is not a common occurrence in all cases is capable of explaining how these obstacles or resistances can be removed. It is not necessary for clear thinking on this subject to know what they are at present, but the only common fact which seems capable of explaining how these natural blocks to fertilization, and the initiation of development could be removed, is at present the increase in the permeability of the ovum. For this reason, without in any way trying to minimize the significance of the facts emphasized by the fertilizin theory, I believe that the permeability change plays a very real part in the process of fertilization, for through it is possible a disturbance of the equilibrium in which we know the egg finds itself prior to impregnation or parthenogenetic initiation.

If this viewpoint is correct, the following picture of both fertilization and parthenogenesis is possible. Fertilizin alone in sufficient concentrations is capable of increasing the permeability of the ovum; smaller quantities when activated by union with sperm are able to exert the same effect. This alteration of the surface may also be brought about by electricity, heat, cold, mechanical shock, specific chemical alteration of the membrane, lipoid solvents, pricking, or any other way of rendering the egg surface more permeable or of accomplishing the same result as an increase in permeability. The immediate effect of this permeability increase is a removal of those obstacles or resistances which constitute the normal fertilization block. These removed, the fertilizin within the egg combines with the egg-receptors, but if fertilizin activated by sperm is present, the egg receptors would combine with the elements for which ex hypothese they have the greater affinity.

So far the facts seem to group themselves harmoniously, but how are we to conceive of the "obstacles" or "resistances"? We might imagine the existence within the unfertilized egg of a body capable of playing the required rôle, but for this assumption there is no evidence. Moreover the substances demonstrated by F. R. Lillie, seem to me sufficient to account for the normal fertilization block if we make a much simpler assumption—one indeed which appears necessary on chemical grounds.

The ovum, according to Lillie, contains egg-receptors, fertilizin, and antifertilizin. Chemical affinities exist between the anti-fertilizin and the spermophile groups as well as between the receptors and the ovophile groups. Although Lillie does not postulate a reaction involving these elements in the unfertilized egg, it is difficult to believe that three substances, demonstrably capable of uniting in the manner indicated, would lie side by side without entering into combination, however loose. If this assumption prove valid, we can consider this union as constituting the normal fertilization block ("resistance," "obstacle") of the unfertilized egg.

From this standpoint it appears possible to explain the block to parthenogenesis in washed eggs. The egg surface prior to fertilization or treatment with parthenogenetic agents, is perfectly permeable to fertilizin. Lillie has shown, however, that the anti-fertilizin can only be removed by destruction of the ova, whose surfaces, with respect to these two substances, appear to act like dialysers. It is quite likely therefore that washed eggs differ from unwashed by a relatively higher concentration of anti-fertilizin. If now fertilizin is permitted to diffuse into a washed egg, it would be bound by the anti-fertilizin and so an initiatory effect, due to its further union (if indeed this occurs[1]) with the egg-receptors would be prevented. According to Lillie's view and the law of mass action, auto-parthenogenesis might occur in washed eggs when the concentration of fertilizin is more than enough to bind the anti-fertilizin. That it takes about two hours to initiate auto-parthenogenesis, and that I failed to initiate it in that length of time in washed ova, are both in harmony with the above suggestion.

So far as I can see, this is the only way at present, in which it seems possible to draw a picture in which all the facts and all the cases may find a place. It follows from the preceding reasoning, as well as more directly from the experiments themselves, that the rôle of the egg secretion (fertilizin) experimentally added to induce development was exactly the same as the rôle of any other parthenogenetic agent, and that it united with the egg receptors, no more than do the electricity, heat, cold, mechanical shock, osmotic pressure, or what-not. On the other hand, like these, the egg-secretion by affecting permeability, brings about conditions under which the ovum is freed from its inhibitors and, in a sense, enabled to fertilize itself. From this standpoint all parthenogenetic methods are methods for inducing auto-parthenogenesis, and fertilization with sperm differs from auto-initiation simply in the fact that the fertilizin which unites with the egg receptors has itself previously united with the sperm in the jelly, or the water about the egg.[1]

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[1] The avidity of fertilizin bound by sperm for the egg-receptors is assumed by Lillie to be greater than in unbound fertilizin. The opposite effect, resulting from a union of the spermophile groups with the anti-fertilizin is not excluded.

SUMMARY.

1. The egg-extracts as well as the egg-secretions of *Arbacia* and *Asterias*, in addition to characteristic effects on spermatozoa, are permeability increasing agents, and initiate development. For the type of initiation here dealt with I have chosen the term *auto-parthenogenesis.*

2. Auto-parthenogenesis is blocked in *Arbacia* by washing the eggs in sea-water.

3. A group "Purple X" which may be split off from the sperm of *Arbacia*, and which also appears in boiled egg-secretion, blocks parthenogenesis in low concentrations and fertilization in high concentrations.

4. Prevention of parthenogenesis by washing can hardly be the result of an absence of fertilizin; prevention by Purple X may be the result of occupancy of the egg-receptors.

5. It is not unlikely that all cases of parthenogenesis are in reality instances of auto-parthenogenesis.

6. With certain minor modifications it appears possible to

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[1] According to F. R. Lillie's account, the sperm unites with the fertilizin in the egg. This however seems incredible, for the affinity between sperm and fertilizin is very great, and since the egg jelly, through which the sperm must pass on their way to the ovum is heavily charged with the fertilizin, it is difficult to see how any unbound sperm could reach the egg. This change in the geographical location of the sperm-fertilizin reaction however makes absolutely no difference in essentials.

[illegible] COLLECTION

14

On the Mechanism of Morphological Differentiation in the Nervous System

I. The Transformation of a Neural Plate Into a Neural Tube

OTTO C. GLASER

From the Zoological Laboratory of the University
of Michigan

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THREE FIGURES

I. INTRODUCTION

The early development of the vertebrate nervous system is among the commonplaces of descriptive embryology. Every elementary text-book tells us that the first clearly recognizable rudiment is a flat patch of cells ectodermal in nature; how in due course of time after the appearance of a longitudinal furrow, the edges of this plate rise, meet in the mid-dorsal line, and fuse to form a tube, enclosing the neurocoel. What however are the forces at work when the primitive plate changes into a tube?

This question was in the mind of His[1] when he wrote his classic letters, "Unsere Körperform." In the fourth of these lucid epistles, His shows that the nervous system, during the period of folding, grows faster than the surrounding tissues with which it is continuous. On the assumption that these resist any increase in the width of the neural plate, he shows that the latter, must fold under the mechanical necessities of the case. Models, in which the entire process could be simulated at will, helped to emphasize the argument.

The well-known experiments of Roux,[2] however, proved that this view of the origin of the neural groove is mechanically impossible, despite the inequalities of growth emphasized by His.

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[1] Unsere Körperform, und das Physiologische Problem Ihrer Enstehung. F. C. W. Vogel, Leipzig, 1874.

[2] Die Entwicklungsmechanik. W. Engelmann, Heft 1, Leipzig, 1905.

Roux indeed was able to produce a fold in the neural plate of the chick by pressure from the sides, but when this pressure was released, the plate instantly returned to its original flat condition. Pressing upon it through the lateral extra-neural membranes, these instead of transmitting the pressure, collapsed, a result which might have been foreseen when their thickness is compared with that of the plate (loc. cit., p. 45).

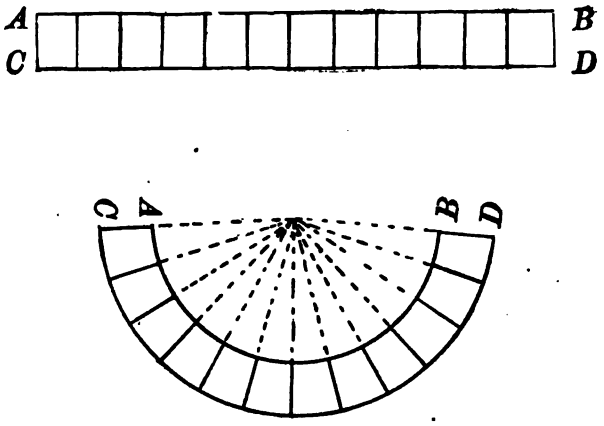

Figure 1

Most conclusive have been Roux's isolation experiments. By cutting the neural plate from its surroundings he found that it still folded in the normal manner, "und zwar geschah dies noch rasher als es normalerweise der Fall ist" (loc. cit., p. 451). Folding occurred even when in addition to lateral isolation, the neural plate was cut transversely into a number of segments. From these experiments the conclusion was drawn that the nervous system is self-differentiating.

Not only do the lateral membranes, as implied in the remark quoted, contribute nothing toward converting the plate into a tube, but they are actually an hindrance, for they exert a pull away from the mid-dorsal line where fusion of the neural folds finally takes place. I have convinced myself by a few simple experiments on the embryos of Amblystoma punctatum, that such pull away from the median axis actually exists. In these, a longitudinal cut be made in the neural plate, the wound pes widely for twelve hours or more if the extra-neural ecto-m has been uninjured, but if this is also cut on each side of e nervous system parallel with the incision in the plate, the und in the latter after twelve hours, is very much smaller n in the first experiment, or has practically disappeared.

f we accept, as it seems to me we must, Roux's conclusion t the nervous system is self-differentiating, we must ask what s statement means. Evidently for the stages of development ler consideration here, self-differentiation is identical with -folding. The question to be answered therefore is how the ral plate can autonomously fold itself.

THE CHANGE IN THE SHAPE OF THE CELLS DURING FOLDING

Rhumbler's[3] very complete analysis of the geometrical relations invaginate gastrulation finds its application to the case in nd. For the sake of simplicity let us imagine it possible to through a neural plate a rectangular cross-section, *ABCD*, which each component cell is itself a rectangle. If this section folded symmetrically about an axis, *AC* and *BD*, and all l-boundaries parallel with them, will radiate toward a center, d *AB* and *CD* will become respectively the circumferences of o spheres, one with the radius, *R*, the other with the radius, + *AC*. It follows from the geometrical necessities of the e, not only that circumference *CD* is greater than *AB*, t that each cell, from being a rectangle, has become a trazium whose lower boundary is greater than its upper.

Although actual conditions in the nervous system are more nplicated, nevertheless, sections through the two stages under nsideration approximate the ideal case very closely. An mination of any section, or any one of the thousands of pubned figures, will disclose many cells in which the change in pe here emphasized is strikingly shown. Indeed, being a metrical necessity, the case cannot be otherwise, but whether change in shape is the result of folding, or folding the result

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Zur Mechanic des Gastrulationsvorganges; Arch. f. Entwicklungsmech., Bd 14.

of the change in shape, remains unanswered. One thing however appears certain; we must seek the answer in the nervous system itself, for the neural plate is self-folding.

III. THE NUMBER OF NUCLEI IN COMPARABLE SECTIONS OF THE NERVOUS SYSTEM AT THE BEGINNING AND AT THE END OF THE FOLDING PROCESS

In order to discover how the neural plate folds itself, we must first find what rôle, if any, cell-division plays in the process. Determinations capable of answering this question could be made if we could count the number of cells in comparable sections at different stages in the development of the tube. However, the nervous system is so largely syncitial in nature, and cell-boundaries, even where present are often so ill defined, that this direct method would surely lead to error. Another road is open however, for we may count accurately the number of nuclei. For this purpose the most satisfactory material I have found are some embryos of Cryptobranchus allegheniensis, for the possession of which I am indebted to Prof. Bertram G. Smith of the Michigan State Normal College.[4]

The period of folding was arbitrarily divided into three, beginning with the flat neural plate, and ending with the neural tube just prior to fusion. Between these extremes is the half-folded plate. For the stages in question I selected ten comparable but unconsecutive sections from the middle point of each series. The sections were all 10 micra in thickness, and the number of nuclei in each was carefully counted. The results are given in table 1, in which each column is headed by a diagram representing the stage of development referred to. Examination of this table indicates that during folding the number of nuclei, and hence of cells per section, does not increase. At the beginning of the period there are, on the average, 62 cells per section; in the middle, 61, and at the end, 59.

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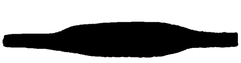
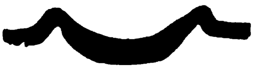
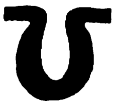
[4] For details on the development of Cryptobranchus, not dealt with in the present paper, see the excellent communications of B. G. Smith: Biol. Bull., vols. 11 and 26, and Journ. Morph., vol. 23.

Naturally these values are not absolute. Lack of uniformity in the distribution of nuclei in the syncitial system is responsible for considerable variations in individual sections, and may have affected the averages. Some errors no doubt have crept in on account of faulty enumeration, and also because the sections are necessarily from different individuals. The seriousness of the first source of uncertainty seems to me largely offset by the remarkable constancy of the averages; against the second source of error I guarded by reflecting the nuclei on paper, dotting each one as counted, and then recounting the dots as a check; the third difficulty was met as completely as possible by choosing embryos from eggs of uniform size laid by a single female.

TABLE 1

Number of nuclei in comparable sections

	STAGE I	STAGE II	STAGE III
			
	63	56	55
	53	64	60
	58	50	73
	69	56	47
	72	50	69
	58	82	59
	59	70	64
	58	74	51
	58	58	52
	68	51	55
Ave.	62	61	59

The conclusion that cell division or better, multiplication, does not occur during the process of folding, although possibly correct, cannot be drawn without a certain reservation, for the nervous system increases in volume during this period (see Section V). With this fact in view one cannot consider the constancy in the number of nuclei per section sufficiently conclusive to warrant the statement that cell multiplication does not occur during the period of folding, and hence can play no part in transforming the neural plate into a tube. However if it does occur, the number of nuclei produced in this way is too small to overcome the 'nuclear dilution' brought about by the volumetric increase of the system as a whole. Relatively, therefore, even if not actually, the number of cells per section remains constant, and we can make no great mistake by assuming that the rôle of cell-multiplication is practically negligible.

IV. THE DISTRIBUTION OF NUCLEI IN COMPARABLE SECTIONS OF THE NERVOUS SYSTEM AT THE BEGINNING AND AT THE END OF THE FOLDING PROCESS

If we mark off a series of points midway between the upper and lower surfaces of the neural plate, a line connecting them will divide the nervous system into an upper and a lower zone. A corresponding line drawn in the half, or fully, folded stages, gives us inner and outer zones. Since morphologically upper and lower are identical with inner and outer respectively, it will be advantageous to use the latter terms also for the unfolded neural plate.

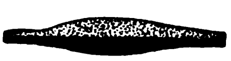
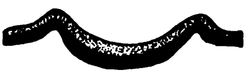
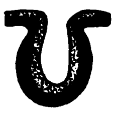
The nuclei of the sections which served as the basis for table 1, are distributed in the inner and outer zones in the proportions given in table 2. Comparing the three stages, we find that the distribution of nuclei in the inner and outer zones is quite different at the beginning and at the end of the folding, and that Stage II occupies an intermediate position. Roughly, the inner zone looses one-half its nuclei and the 'nuclear concentration' in the outer zone increases by this amount. Absolute correspondence between the nuclear loss of one zone, and the gain in the other cannot be expected. Not only must we recall the sources of error mentioned in section III, but we must also remember that the division into inner and outer zones is a somewhat arbitrary expedient, and furthermore that the neural plate, although spoken of as flat in Stage I, is in reality quite irregular in detail, and moreover exhibits, more or less, the general curvature of the sphere of which it is a part. Without attempting any special refinements which seem to me quite unnecessary, it is obvious that during folding there is a marked outward migration of nuclei (see text-figure 2). With this outward migration, there is associated, from the geometrical necessities of the case, a distinct increase in the volume of the outer zone. Both these changes in the folding nervous system would occur if this tissue were suitably pressed upon from without, but since the neural plate folds itself, the forces that result in the translocation of materials and the change in the shape of the cells must be sought within the autonomous system.

V. THE INCREASE IN THE VOLUME OF THE NERVOUS SYSTEM DURING FOLDING

Change of shape in the cells of a folding tissue may occur with constant volume. In the neural plate, for instance, the inner zones of the cells might decrease by an amount exactly equalled by the increase in the outer zones. This, however, is not true.

TABLE 2

Distribution of nuclei in inner and outer zones

STAGE I		STAGE II		STAGE III	
					
Inner	Outer	Inner	Outer	Inner	Outer
32	31	31	25	15	40
32	21	22	42	15	45
34	24	16	34	21	52
55	14	27	29	13	34
39	33	18	32	22	47
38	20	27	55	16	43
31	28	33	37	26	38
33	25	29	45	13	38
37	21	21	37	20	32
44	24	19	32	20	35
Ave. 38	24	24	37	18	39

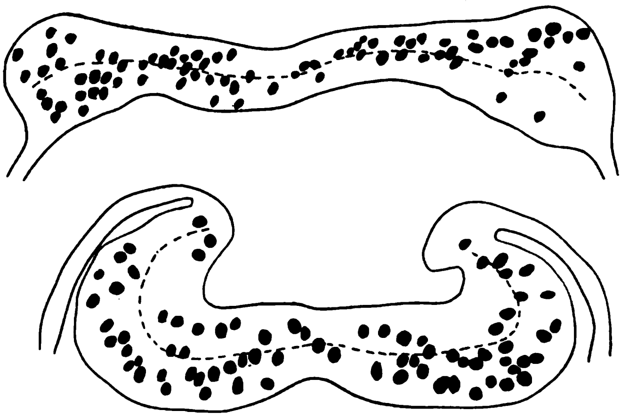


Fig. 2 Two sections through the embryonic nervous system of Cryptobranchus allegheniensis, showing the nuclear distribution in Stages I and II. The sections are from the same series and regions as those dealt with in the tables but contain for Stage I, six, and for Stage II, one nucleus more than the maximal number recorded in table 1. In the unfolded plate there are in the present case, 78 nuclei, of which 47 are in the inner zone, and 31 in the outer; in the half-folded plate, there are 75 nuclei, 21 in the inner zone, and 54 in the outer. Nuclei which happen to fall on the line separating the two zones are ascribed to the one into which the greater portion of their mass projects.

Adequate measurements cannot be carried out directly. Instead, we may compare the areas of the two regions, in section, for area is definable as volume in one plane. On this basis, determinations capable of giving some insight into the volumetric relations during involution are easily made; all that is needed is to draw the sections at constant magnification with the aid of a camera lucida, and then by means of a planimeter, trace the relative areas of the inner and outer zones. The results of such measurements, carried out on the same sections used in making the nuclear counts, are presented in table 3. From this table it is apparent that the areas of the two zones are related during the process of folding by the following ratios:

	STAGE I	STAGE II	STAGE III
Inner zone	4.8	4.3	7.4
Outer zone	4.4	6.8	12.3

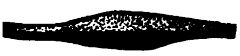
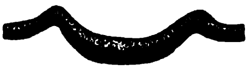
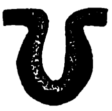
n other words, while inner and outer zones are approximately al in Stage I, in Stage II, the outer zone exceeds its original by half, and the inner remains practically unchanged. In ge III on the other hand, both zones show an increase, but inner, roughly, has doubled, the outer, trebled, its volume. hough it is impossible under these circumstances to determine r much of the increase in the volume of one zone is due to the ration of nuclei and cytoplasmic materials from the other, y no means follows that the outer zone does not gain at the ense of the inner. Indeed such translocation is definitely ved in the case of the nuclei, and appears inevitable for the er cell contents.

VI. ON THE CAUSE OF THE INCREASE IN VOLUME

ince cell-multiplication during the process of folding appears ligible, it cannot be concerned practically with the increase olume which takes place at this time. It follows that the s of the nervous system must individually increase in volume,

TABLE 3

Relative areas of inner and outer zones

STAGE I		STAGE II		STAGE III	
					
Inner	Outer	Inner	Outer	Inner	Outer
4.6	4.8	5.3	8.4	6.6	10.9
4.4	3.7	4.1	7.2	6.9	12.6
6.7	3.9	4.6	6.8	6.9	12.6
5.2	4.7	4.3	5.8	7.8	12.8
5.0	4.1	4.1	6.0	7.3	13.2
4.8	4.8	4.0	6.9	6.8	11.3
4.6	4.5	5.0	6.5	7.7	11.3
3.3	4.9	4.2	7.8	7.7	10.8
4.4	3.9	3.7	6.5	7.3	11.6
4.9	5.0	3.8	6.3	8.5	10.3
ve. 4.8	4.4	4.3	6.8	7.4	12.3

and this might be due to the absorption of water. Such absorption would be capable of direct demonstration if it were possible to compare the water content of the neural plate with that of the neural tube, but very serious obstacles of a technical nature stand in the way of the required determinations. It would be exceedingly difficult to isolate neural plates in sufficient numbers, or with sufficient accuracy, to make the necessary weighings reliably. Another method of procedure is open.

The rate of growth in these early stages of the nervous system is known to differ from that of the surrounding tissues. Since growth in general is measurable in terms of water absorption it follows that the differential growth of the neural plate and tube must be the reflection of a differential absorption of water on the part of their component cells. If this is correct then at the stage of complete involution, the water content of the neural tube should differ from the water content either of the larva, taken as a whole, or of certain portions, for unless this were true, the embryo, instead of having grown in complexity, would simply have grown in size.

The most satisfactory material available for the study of this question proved to be the eggs of the frog, Rana pipiens, and of the salamander, Amblystoma punctatum. Development in both cases was allowed to proceed normally until the bodies of the embryos could be conveniently cut from the yolk-sac, an operation easily carried out with a minimal loss of tissue. Unfortunately the separated portions cannot be further divided, and even if the isolation of their constituent layers were possible the inclusion of yolk within the cells of the nervous system would leave an uneliminated and unavoidable error. However it is safe to assume that our operation results in the separation of two tissue masses, one predominantly yolk, the other predominantly nervous.

The fresh weight of the entire larvae as well as that of the separated tissue masses was in each case ascertained after carefully removing the superficial water. Following this, dry-substance determinations were made in the usual manner by complete dessication at 60°C. *in vacuo*, over P_2O_5. For the sake of easy comparison, all the results are assembled in table 4. From this table it is at once apparent that the water content of the frog and salamander embryos at this stage of development belong to the same order of magnitude, and further that the water content of the yolk-sac of the frog larvae is not very different. Corresponding determinations for the yolk-sac of Amblystoma proved impracticable as the consistency of the yolk in these embryos is such that considerable losses occur when the sac is removed. However, the values for the entire larvae are identical in the two cases.

Comparing the embryonic nervous systems of these two forms with the entire larvae or the yolk-sacs, it is seen at once that the water content of the first belongs to a totally different order of magnitude, for the nervous system is a tissue which, within the limits of error may be said to contain 80 per cent of water and 20 per cent of dry substance.

TABLE 4

Showing water content four to five days after fertilization

MATERIAL	FRESH WEIGHT	DRY WEIGHT	DRY SUBSTANCE	WATER
R. Pipiens	*grams*	*grams*	*per cent*	*per cent*
38 Larvae	0.1278	0.0557	43.~~8~~6	56.~~2~~4
50 Larvae	0.1718	0.0722	42.0	58.0
39 Larvae	0.1815	0.0~~6~~730	40.2	59.8
Average			**4~~2~~1.~~0~~9**	**58.~~0~~1**
24 Yolk-sacs	0.0440	0.0204	46.~~5~~4	53.~~5~~6
31 Yolk-sacs	0.0585	0.0264	45.~~2~~1	54.~~8~~9
Average			**45.8**	**55.2**
24 Nervous systems	0.0464	0.0098	~~19~~21.1	~~80~~78.9
31 Nervous systems	0.0714	0.0149	20.~~8~~9	79.~~2~~1
Average			**21.0**	**79.0**
A. Punctatum				
16 Larvae	0.0955	0.0399	41.8	58.2
15 Larvae	0.0992	0.0406	40.9	59.1
Average			**41.4**	**58.6**
125 Nervous systems	0.3914	0.0785	19.9	80.1
52 Nervous systems	0.1756	0.0400	22.8	77.2
15 Nervous systems	0.0524	0.0106	20.2	79.8
69 Nervous systems	0.2039	0.0363	17.8	82.2
Average			**20.2**	**79.8**

The essential correctness of these values for the nervous system is guaranteed by the identity of the averages, the relative constancy of the individual observations, and finally by comparison with the adult condition. This comparison[5] can be made in the case of Rana pipiens, because Donaldson[6] has given us certain standard values for this form. In making this comparison it is more correct to use the water content of the adult cord, for this is less differentiated than the brain and hence more nearly in the larval condition. According to Donaldson's figures (loc. cit.) the average water content of 12 cords of Rana pipiens is nearly 80.5 per cent, a value identical with mine of 80.1 per cent for the larval system.

Since the rate of cell-multiplication during folding is either zero or practically negligible; since this period, moreover, coincides with a great increase in volume, and finally, since the water content of the neural tube differs so radically from that of either the yolk-sac or the larva taken as a whole, it seems scarcely doubtful that the differential absorption by the nervous system occurred during the process of involution. This, however, does not prove that the differential absorption is responsible for the folding.

VII. ON THE THEORY OF FOLDING

Since every metazoan body arises from germ-layers having a common origin in the egg, and connected with one another without interruption, it follows that even a complicated organism, theoretically at least, could be unravelled and spread out in the form of a continuous membrane. It has been evident for many years that in embryogenesis, the commonest occurrence leading to increased complexity of form, is the folding process, but its causes have not been adequately analysed, nor have attempts at analysis received the recognition from anatomists and embryologists, which they deserve.

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[5] Otto Glaser, The water content of the embryonic nervous system. Science, vol. 39.

[6] Donaldson, Henry H., Further observations on the nervous system of the American leopard frog, Rana pipiens, etc. Jour. Comp. Neur., vol. 20; see also numerous earlier papers.

From the standpoint of static morphology, the analysis of an organism into a system of folds may appear satisfactory enough, and constitutes a step forward. From the same standpoint, however, every fold is like every other, a complication, the contemplation of which can give us no idea as to how it was produced. From the dynamic point of view, the mode of production is the mportant thing, and a moment's reflection is enough to show that significance of one fold may be quite different from that of another.

Experimental analysis of the early stages of the nervous system has gone far enough to show that here the folding process is autonomous. The same effect could be achieved by coercion, and would be morphologically indistinguishable. But coercion is a very different process from the one under consideration, and certain foldings of the heart and digestive system in the embryo, have only a superficial resemblance to the autonomous olding of the nervous system. Nevertheless, we are not without analogies, for it follows from Rhumbler's work (loc. cit.) that nvaginate gastrulation belongs to the same category.

In the first place, Rhumbler draws attention to the significance of the change in shape undergone by the entoderm cells during infolding. Before as well as after gastrulation the cells are wedge-shaped, but in the blastula, the narrow ends of the wedges point inwards, as they do in all the other cells of the spherical larva, whereas during and after invagination the wedge-shape of the entoderm cells is completely reversed and their narrow ends now point outward. This change in shape, which has also been emphasized by Conklin,[7] occurs, as we have seen in the nervous system. According to Rhumbler, a translocation of materials within each of the involved cells is a mechanical necessity. "Die Umgestaltung der Zellen der Entodermplatte, d. h. die Verbreiterung der Entodermzellenkeile auf der Blastocölseite, *erfordert unbedingt Zellensubstanz-verlagerung innerhalb jeder einzelnen Entodermzelle nach der Blastocölseite hin, um verbreitern zu können was vorher zugespizt war*" (loc. cit. p. 432).

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[7] Mosaic development in Ascidian eggs. Jour. Exp. Zoöl., vol. 2, p. 163.

The migration of the nuclei which I have described is in strict harmony with this idea, and if the nuclei migrate it is probable that other portions of the cell contents also undergo a change in location.[8] The occurrence of the same changes in shape on the part of the infolding cells in the two cases, however, is in itself not enough to show that invaginate gastrulation and the formation of a neural tube are fundamentally identical, for gastrulation by coercion would force upon the entoderm cells the shape which they would assume autonomously, if invagination were an autonomous process. There are cogent reasons for believing that this is true.

According to Rhumbler (loc. cit., p. 410) there are conceivable three ways in which the entoderm cells might possibly be coerced from without. In the first place, differential growth on the part of the ectodermal and entodermal elements of the blastula might result in invagination; in the second place, the blastula, growing inside the egg-membrane, might have invagination forced upon it if the entodermal plate were less resistant to mechanical pressure than the ectoderm; and finally, the decrease in the volume of the blastocoelic fluid during invagination might result in a suction which would be followed by a caving-in of the weakest region in the wall.

The second and third possibilities are readily disposed of. It is only necessary to recall that invaginate gastrulation takes place perfectly well in the absence of an egg-membrane (Rhumbler). Furthermore, the entodermal plate cannot be the weakest region in the wall of the blastula since in general its component cells are the largest which the larva possesses. Mechanically the relation between the entoderm and the ectoderm of the blastula, must be the same as that between the neural plate and the extra-neural membranes. Suction due to a decrease of the blastocoelic fluid would bring about the invagination not of the entoderm, but of the ectoderm.

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[8] In this connection, figure 2 in a recent paper by J. F. Gudernatsch in The Anatomical Record (vol. 7, p. 416) is interesting. In this picture the nuclei are located in the inner ends of the gastral plate cells *before* invagination. The author imagines that this localization renders the inner ends of the cells less compressible than the outer, and that invagination is caused by pressure exerted by the ectoderm on the more compressible outer ends of the gastral cells. It seems curious that the work of Rhumbler and Roux, mentioned in the very extensive bibliography attached to this paper, should have made so little impression.

Differential growth as a factor in invagination is not so easily disposed of. Rhumbler's analysis deals with the following possibilities:

Case I. Cell multiplication in the ectoderm proceeds at a higher rate than in the entoderm. The result is pressure upon the entodermal plate from the periphery, and this pressure may be sufficient to bend the plate. However, not only is this region of the blastula the least likely of all, to give way, but as long as the broad ends of the entodermal wedges point outward (Rhumbler) any folding produced by the means imagined would necessarily result in evagination, not invagination.

Case II. Cell multiplication in the entoderm proceeds at a higher rate than in the ectoderm. The effect would be the same as in Case I. A pressure on the entodermal plate would result, but no matter how produced, the plate could not fold inwards.

Consideration of these two possibilities of differential growth is worth while as an aid to clearing the ground, although Case II was unnecessary even at the time that Rhumbler wrote, for Morgan[9] had found that during gastrulation

> Karyokinetic division is no more frequent in the cells involved than elsewhere. At the end of the period, if the posterior hemisphere be examined, it will be found that the plate of cells has disappeared from the surface, and that the surface nuclei are little more frequent than the surface nuclei of the anterior hemisphere. Karyokinetic division therefore plays no part in the development of the archenteron of the gastrula of Sphaerechinus (loc. cit., p. 85).

Although my conclusion with regard to the rôle of cell-multiplication during the folding of the neural plate is stated more conservatively, Morgan's result with respect to the same factor in the invagination of the gastral plate, is practically identical.

With respect to the change in shape, the consequent translocation of cell-contents, the influence of external pressure, and finally, the rôle of cell-multiplication, invaginate gastrulation is identical with the folding process undergone by the nervous system. We may, therefore, safely attribute to the gastral plate an equal degree of autonomy.

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[9] Studies of the 'partial' larvae of Sphaerechinus. Arch. f. Entwicklungsmech., Bd. 2.

But the elimination of cell division does not necessarily eliminate differential growth. We have already seen that an increase in the volume of the nervous system takes place at the time of folding, and that this growth, occurring with a negligible rate of cell-multiplication, is the result of water absorption. If such absorption were demonstrable for the gastral plate we should have one more point of identity between the two processes under comparison.

Unfortunately I have no trustworthy direct measurements. Nevertheless, there are considerations which seem to bear closely on the point at issue. Thus Morgan (loc. cit., p. 85) says:

> The nuclei in the walls of the archenteron enlarge during the invagination period and lie further apart than they did in the plate. This might give us some clue as to the mechanical principles involved in the process if we could estimate the volumes of the cells (protoplasm) before and after the process, but this it is impossible to do.

I have little doubt that the methods which I have employed for detecting the change in volume during the folding of the neural plate will be applicable to the process of invaginate gastrulation. What is of more immediate concern, however, is the increase in the volumes of the nuclei, and the fact that after invagination they "lie further apart than they did in the plate."

Morgan proved that there is no increase in the number of entoderm cells during invagination. The only factor which can be concerned in separating the nuclei is an increase in the volume of the extra-nuclear material. If such increase were the result of water absorption one would expect the increase in the size of the nuclei which was observed.

While this reasoning may be valid, nevertheless it is desirable to know whether nuclear size is a reliable index of the relative amount of water which the cell contains. That this actually is the case is indicated by certain measurements which I have made on the unfertilized ova of Asterias forbesii.[10] These eggs constitute a very favorable material for the solution of this problem, not only on account of their size and shape, but also because their nuclei (germinal vesicles) are easy to see in the living cells and like these are spheres. In table 5 are given the diameters of eggs and nuclei which were first measured in normal sea-water, and afterwards in hypotonic.

From the figures in table 5 it is at once apparent that the cells absorb water in the hypotonic solution, and moreover that when they increase in volume, their nuclei do so likewise. It follows from this that the volume of the nucleus may be taken as an index of the relative amount of water held by the cell, and that the increase in the size of the nuclei noted by Morgan during gastrulation, indicates an increase in the water content of the gastral plate. In this tissue, therefore, we have differential growth just as in the nervous system, and instead of being the outcome of the synthetic processes involved in growth by cell-multiplication, the increase in bulk is here also the result of water absorption.

TABLE 5

Showing relative diameters of Asterias ova and their nuclei. The hypotonic sea-water was made by diluting six volumes of the normal sea-water, with four volumes of distilled water. The units of measurement are the same in all cases, but the exact absolute value cannot be given at this time. With greater as well as with smaller dilutions measurable changes in the same sense can be detected

		NORMAL SEA-WATER	HYPOTONIC SEA-WATER
		units	*units*
Lot I	47 eggs	152.0	188.0
	47 nuclei	0.7	0.8
Lot II	49 eggs	154.0	170.0
	49 nuclei	0.7	0.8

The question as to the rôle of this absorption in the process of folding remains to be answered. As far as invaginate gastrulation is concerned, so long as the entoderm cells continue to have the shape they possess in the blastula, an increase in volume could result only in stretching the ectoderm, and bending the gastral plate outward. If the increase in volume were to continue, the blastula would flatten, and finally the ectoderm, unable to stretch further would tear away from the entoderm. In the nervous system the situation would be exactly the same. The differential growth of neural and gastral plates alone therefore could never convert these structures into tubes, a conclusion reinforced by Roux's isolation experiments, for when a neural plate, completely isolated from other tissues folds itself, differential growth is certainly excluded. What significance then are we to attach to the water absorption, and how are we to explain the folding?

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[10] For an account of the methods employed see, Glaser, The change in volume of Arbacia and Asterias eggs at fertilization. Biol. Bull., vol. 26, p. 84.

Rhumbler (loc. cit.) has pointed the way to an answer. The cells of the gastral and neural plates change in shape during invagination and folding. Since both processes are autonomous, an explanation would be found if we could show how the cells of themselves could change in form so that no other arrangement except that of the folded or invaginated state is possible. The factor possessing the necessary requirements, is, according to Rhumbler, differential surface tension.

Three cases are considered, as follows:

Case A. In the spherical blastula, all the cells are wedges whose smaller ends point toward the center. The outer poles of the entoderm cells are bathed by an external medium of relatively constant composition; the inner poles on the other hand project into the blastocoelic liquid whose composition is not only different, but no doubt changes during the course of development. For instance, the concentration of CO_2 alone must be greater in this liquid than in the external medium which not only has a far greater volume, but into which ciliary convection currents are constantly driving the CO_2 from the surface of the larva. Now the surface tension of a cell depends upon the nature of the surface and this is determined equally by the characters of two media which it separates. Rhumbler imagines that in the gastrula we have conditions under which the tension of the inner surfaces of the entoderm cells may well be different from that of the outer, and if it were less, then the outer surfaces with their higher tension would, like elastic bags, squeeze the cell-contents inward. The inner surface under the circumstances would give way until an equilibrium resulted in which the entodermal wedges would be reversed. Theoretically such reversal is all that is needed to insure invagination or folding.

But why does this not apply equally well to the other cells of the blastula? Why do the entoderm cells alone invaginate? For the sake of simplicity, Rhumbler (loc. cit., p. 448) considers first the mechanical conditions which may be supposed to obtain if the surfaces of the ento- and ectoderm cells are identical in physico-chemical composition. From the greater size of the entodermal elements it follows that their surface tension is less than that of the smaller ectoderm cells.[11]

If now the blastocoelic fluid lowers surface tension, it would bring about, per unit area, an equal lowering in all the cells, but in the entoderm, because of its absolutely greater surface, the absolute lowering would exceed that in the ectoderm.[12]

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[11] The phenomenon of exo-gastrulation in lithium larvae (Herbst, Arch. f. Enwicklungsmech., Bd. 2, p. 455, referred to by Gudernatsch, loc. cit., p. 417) harmonize with the opinions of Rhumbler as expressed here, for in these embryos all the blastomeres are swollen and vacuolated, but just before exo-gastrulation the entoderm cells decrease in volume and become actually smaller than the ectoderm. This experimental fact harmonizes equally well with the somewhat freer interpretation which I shall present later on. In this connection it is interesting to recall the 'exo-neurula' of the frog, also produced by the use of lithium (Hertwig, Morgan). Gudernatsch (p. 423) also refers to Driesch's observation that gastrulation occurs in Sphaerechinus blastulae (Mitteil. Zool. Stat. Neapel, Bd. 11, p. 221), no matter whether they are derived from the micomeres or macromeres of the early cleavages. This fact does not preclude minute differences in the size of the cells in these 'partial' larvae, nor necessarily even differences in their chemical composition.

[12] The passage in Rhumbler (p. 449) in which this matter is discussed, reads as follows: "Die Entodermzellen sind aber grösser und haben desshalb auch eine 'absolute' grössere Oberfläche, so dass bei ihnen die Spannungserniedrigung wohlgemerkt 'pro-Einzelzelle' viel erheblicher ausfallen muss als bei den einzelnen Ectodermzellen, zumal die kleineren Ectodermzellen (weil sie von Haus aus eine grössere Oberflächenspannung pro Flächeneinheit besitzen), an sich zur Erziehlung des gleichen Effektes nicht die gleiche, sondern eine entsprechend beträchtlichere Herabminderung der Oberflächenspannung pro Flächeneinheit verlangen würden, während ihnen doch nur eine gleiche geliefert wird." Gudernatsch (loc. cit., p. 419) writes: "Rhumbler however believes that chemotaxis alone is the inducing factor of gastrulation."

Under these circumstances the entodermal cells would move into the blastocoel before the ectoderm, and the rise in internal pressure owing to the incompressibility of the blastocoelic fluid would render impossible any immigration or invagination on the part of any other cells.

Case B. The conditions of Case A are needlessly difficult, and were assumed simply to show that invagination is conceivable from this standpoint even under the most adverse circumstances. But the physico-chemical constitution of the surfaces of neither the gastral nor neural plate cells can be identical with that of the neighboring ectoderm for they do not enclose identical chemical systems. This is indicated not only by the localization of yolk and 'organ-forming' substances in the entoderm, and by the well-known cytological specificity of the early nerve cells, but especially by the specific water-holding capacity of the embryonic nervous system,[13] a specificity, identical with that of the adult system, and conceivable only as the outcome of a specific physical-chemical organization.

Rhumbler, for similar reasons, considers as a second possibility, "dass die *Zelloberflächen der sich einstülpenden Zellen so stark anders beschaffen wären* als diejenigen der sich nicht einstülpenden Ectodermzellen" (loc. cit., p. 450), that only the former react toward the blastocoelic fluid in the manner necessary to insure invagination.

Case C. Finally Rhumbler suggests that the greater size of the entoderm cells and their greater readiness to react appropriately to the influence of the blastocoelic fluid, might coöperate, and together bring about the decrease in surface tension required for the change in shape.

Although Rhumbler seems to me to place the emphasis where it belongs, certain qualifications appear to be either necessary or pertinent. In the first place, while the incompressibility of the blastocoelic liquid follows necessarily from the physical properties of fluids in general, it cannot be employed to explain in Case A and subsequent cases, why the ectoderm cells fail to invaginate, for if the fluid remained within the blastocoel, its incompressibility would of course render the invagination of the entoderm cells equally impossible.

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[13] See Glaser, The water-content of the embryonic nervous system. Science, vol. 39, p. 730.

In the second place, the explanatory value of 'surface tension' does not seem to me acceptable without certain reservations. That it may be the important factor, cannot be denied, neither can its importance be considered as demonstrated. Surface tension, together with the Gibbs-Thompson principle, gives an adequate physical explanation for the concentration of certain substances in the surface of the cell, but inasmuch as the substances so concentrated undergo changes in aggregation resulting in the formation of solid films, the application of the laws of surface tension meets with some difficulties. As long as we have no clear conception of the order of magnitude of the 'surface tension' of cells, and moreover lack really adequate methods of measurement, it seems rather questionable to lay too much emphasis on this particular factor. As Loeb[14] has pointed out, even in the relatively simple cases of amoeboid movement, mere changes in surface tension hardly seem adequate, for

> If it is true that the Amoeba is covered with a solid film, one condition for the formation of a pseudopodium must be a local liquefaction of protoplasm. In consequence of such liquefaction new protoplasm must flow out, which subsequently will form a new solid film at its surface. This may again be liquefied, and a new streaming may occur, etc. Such liquefactions can be caused by lack of oxygen . . . .; but they may also be caused by other chemical changes. I am inclined to believe that phenomena of liquefaction play at least some rôle in the processes of protoplasmic motion.

If such liquefactions occur in the neural and gastral plates, they, rather than alterations in surface tension, might be the important factors.

Until the possibilities have been experimentally limited or defined, it seems unwise to specify too carefully which particular surface effect, or combination of surface effects, is responsible for the change in shape undergone by the cells in the folding plates.

[14] Jacques Loeb, The dynamics of living matter, p. 57.

Nevertheless, even without this specificity, desirable as it would be, it is possible to apply Rhumbler's general thesis to the folding of the neural plate.

Let *ABCD* (fig. 3) represent a cell in the neural plate. Along the two sides *AD* and *BC* the cell is bounded by others like itself. The side *AB* limits the system toward the external world, the side *DC* toward the internal, but nevertheless extra-neural, environment. The shape and position of the cell is the expression of a state of equilibrium which depends not only on the nature of the physical-chemical system, *ABCD,* but equally upon conditions outside.

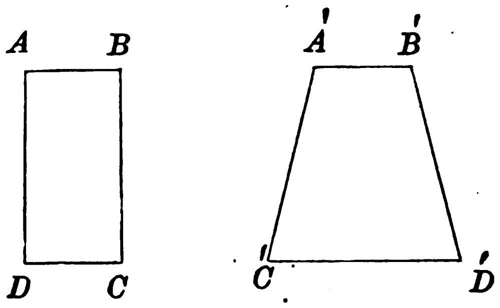

Figure 3

In the neural tube the shape of this cell is represented by *A′B′C′D′*, and this also is the expression of an equilibrium dependent on internal and external conditions. Obviously, however, since the shape and perhaps also the position of the cell differ from those it originally had, the new equilibrium is not indentical with the old. Now the chances of a disturbance of equilibrium along the lines *DA* and *CB*, are not very great for the cell is bounded on these sides by chemical systems like itself. Along the line *AB* also the chances of disturbance are small, because here the cell abuts upon an external environment whose constancy is relatively high. Along the line *DC*, however, the cell is subjected to influences due to what is going on in the rest of the embryo, and as important changes are constantly occurring within every developing organism, it is certainly not unreasonable to imagine that the internal environment, compared with the external, is relatively unstable. From the morphological standpoint, this is only another way of saying that development is taking place, from the physiological it is almost self-evident, and with reference to one chemical factor, at least, I know it to be true, for whereas *the frog's egg is neutral in its reaction to litmus, the contents of the young larvae, not yet hatched, are distinctly acid.*

Whether the transition from neutrality to acidity, or some other chemical change, is important, certainly the relative inconstancy of the internal extra-neural environment is no assumption. Rhumbler attributes a similar instability to the blastocoelic fluid, and imagines that when certain substances have reached a certain concentration, a lowering of the surface tension in the entodermal plate will result. Correspondingly, if such a lowering should occur in the neural plate, the side AB would lengthen, the cell would assume the shape $A'B'C'D'$, and the observed translocation of intra-cellular contents, and necessarily also the observed folding, would be accounted for.

For reasons already given, I prefer to assume, instead of a lowering of the surface tension, simply 'a surface effect.' This does not exclude the factor emphasized by Rhumbler, but leaves room for such other possibilities as liquefaction, 'etching' and changes in permeability. Any of these, singly or in combination might result in a weakening of the internal surface, and though the effect of such weakening would be identical with that brought about by a lowering of the surface tension the actual mechanism might nevertheless be quite different. But what evidence have we for this 'surface effect'?

A surface effect necessarily involves a change in permeability. and a change in permeability may be followed either by a gain or loss of water.[15]

Since now the nervous system demonstrably, and the entoderm, probably, increase in volume during folding, and since this increase is the outcome, not of cell-multiplication, but of water absorption, a surface effect, involving a change in permeability is practically certain. Since the sense of this change, for water at least is positive, it seems likely that the affected surface would be mechanically weakened by it.[16] With the initiatory changes in folding accounted for on this basis, the possibility that the absorption of water has after all a 'formative' influence, once more arises, for even if an increase in volume cannot of itself induce folding, it might accelerate or retard the process when initiated by other forces.

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[15] Glaser, On inducing development in the sea-urchin (Arbacia punctulata) together with considerations on the initiatory effect of fertilization. Science. vol. 38, p. 446.

A colloidal rod or tube which bends in boiling water can easily be shown not to have a uniform dry substance, and a board may warp through differential water absorption. Since the water must enter the neural plate via the affected surface, a differential localization would greatly facilitate folding.[17]

I have tried many experiments for the purpose of detecting such differences in the intra-cellular concentration of water, but have not succeeded in finding any evidence of differential distribution. Indeed on physical-chemical grounds, such an arrangement of the water does not appear very likely. It is of course still less likely to be distributed so as to oppose the folding. Most probably the distribution is what one might expect, an arrangement, which indeed affects the size of the cells, but has no other formative, or morphogenetic significance at this particular stage of development.

[16] See Höber, Physikalische Chemie der Zelle und der Gewebe, 3rd ed.

[17] In the case of the gastral plate, the increase in size must be due to the absorption of water from the blastocoel. As a matter of fact the blastocoelic fluid decreases in amount, and this decrease is absolutely essential for invagination, not because the absorbed water is differentially distributed in the entoderm cells, or because its removal from the cavity produces a suction, but because in this way an insurmountable obstacle, an incompressible liquid, is removed. The following experiment is not without interest in this connection. Normal free-swimming Arbacia blastulae placed in sea water diluted with an equal quantity of distilled, absorb a great amount of water of which part enters the blastocoel and part remains in the cells themselves. The development of such inflated blastulae is arrested, but if returned at the end of 24 hours to normal sea water they instantly regain their former size, and development once more proceeds normally.

VIII. SUMMARY

We may summarize the foregoing considerations as follows:

1. The folding process by which the neural plate becomes converted into a neural tube, does not depend on coercive pressure from without, and in this sense is autonomous (Roux).

2. The cells of the neural plate, actively engaged in folding undergo the change in shape emphasized by Rhumbler in the invaginating gastral plate.

3. During the period of involution in Cryptobranchus cell-multiplication appears to proceed at a negligible rate, a conclusion practically identical with the inference drawn by Morgan from a study of gastrulation in Sphaerechinus.

4. If the folding nervous system be divided into an inner and outer zone, an outward migration of nuclei during involution is demonstrable. Translocation of intra-cellular contents also occurs in invaginate gastrulation (Rhumbler).

5. During involution no doubt the outer zones increase at the expense of the inner, but the exact extent of this is difficult to determine since the inner zones also increase.

6. This 'growth' of the nervous system is not the result of the synthetic processes ordinarily associated with cell-multiplication, but is the outcome of water absorption.

7. The folded nervous system contains 80 per cent of water and 20 per cent of dry substance; the entire embryo, on the other hand, has only 58 per cent of water and 42 per cent of dry substance. For the isolated yolk-sac of the frog's embryo the corresponding figures are 55 per cent and 45 per cent respectively. During involution, therefore, differential water absorption takes place in the nervous system.

8. Such differential absorption can also be inferred for the entoderm from Morgan's observations on the gastral nuclei of Sphaerechinus, for these increase in volume and lie further apart after gastrulation than before. These changes would be expected if the gastral plate absorbs water at this time.

9. As shown by the behavior of Asterias ova in normal and hypotonic sea water, nuclear size may be used as an index of the relative water content of the cell.

10. The 'morphogenetic' or 'formative' effect of the water absorption is in all probability zero. The size of the cells is of course increased, but such increase can only affect the process of involution if the absorbed water is differentially distributed in the cell. For this there is no evidence, and little probability. The real significance of the water absorption seems to lie in the fact that it is a symptom of a surface effect which involves apparently a change in the permeability of the neural plate cells.

11. The surface affected is more likely to be the one bounded by the extra-neural, intra-embryonic environment than any other.

12. The contents of the frog's egg are neutral to litmus; those of the larva not yet hatched, acid.

13. On the basis of these facts and certain other considerations, it is proposed to modify Rhumbler's theory of autonomous folding by substituting 'surface' effect for 'surface tension.' This does not exclude surface tension from the list of possible factors, but leaves room for others such as liquefaction of the surface, 'etching,' and changes in permeability, all of which are possible in solid films.

14. The surface effect indicated by the absorption of water during folding, may very possibly result in a mechanical weakening from which the involution of the neural, and the invagination of the gastral plates follow, not only automatically, but with the demonstrated autonomy.

POSTSCRIPT

After this manuscript had been completed, I discovered the very recent and important contribution of Gurwitsch, "Der Vererbungsmechanismus der Form" (Arch. f. Entwicklungsmech., Bd. 39, p. 516).

This work falls naturally into two divisions, one theoretical, the other dealing with concrete observations. Inasmuch as the theoretical discussion involves the conceptions of 'Partialzweck' and the 'dynamisch präformirte Form' I must postpone, perhaps indefinitely, the attempt to enter these difficult regions.

With respect to the concrete results of Gurwitsch, it is to be noted that the distribution of the nuclei in the folded regions studied by him, is identical with the distribution I have found. However, there are also significant differences, particularly in connection with the rôle assigned to cell-multiplication and cell-migration, but these 'discrepancies' are not necessarily indicative of errors, instead they may be only the inevitable results of dealing with two quite different periods of development as well as with different materials. The neural plate of Cryptobranchus shows that folding can take place without cell-multiplication, and the conclusion that it does so, is in no wise affected by the frequent occurrence of mitoses in the corresponding stages of other forms. These constitute a less favorable material inasmuch as they do not present the simplest case. However, even these cases may prove to be instructive for the localization of the mitoses in the mammalian neural plate is such that cell-multiplication, if effective at all, would not facilitate, but oppose the process of involution.

I am inclined to hazard the guess even now, that the cell-migrations in the folds studied by Gurwitsch are effects rather than causes, but as I shall approach these stages from another angle, in a forthcoming paper, I shall reserve until that time a full discussion of the bearing of Gurwitsch's basic observations. In the meantime, in order to avoid misunderstanding and anticipate its attendant needless difficulties, I should like to impress on the reader as strongly as possible, that the mechanism of the process so carefully analysed by Gurwitsch, involves factors which are not concerned in the autonomous folding of the neural plate. In the present instance, the assumption that the mechanics of every folding process are identical with those of every other, would certainly lead to erroneous conclusions.

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15

REVIEW OF "BAUSTEINE ZU EINER BIOLOGISCHEN WELTANSCHAUUNG"

BY

OTTO Charles GLASER

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eprinted from SCIENCE, *N. S., Vol. XLI., No. 1052, Pages 324–327, February 26, 1915*]

:EVIEW OF UEXKÜLL'S "BAUSTEINE ZU EINER BIOLOGISCHEN WELTANSCHAUUNG"

usteine zu einer Biologischen Weltanschauung. Von JAKOB, BARON VON UEXKÜLL.
lünchen, F. Bruckmann A.-G. 1913.

7on Uexküll sketches and advocates a vital-
c "Weltanschauung" in eighteen popu-
ized essays, collected for convenience into
r larger groups, the whole, sponsored by
ix Gross, and dedicated to Stewart Hous-
Chamberlain. "Die Neuen Probleme" is
roductory; "Der Neue Standpunkt" in-
des discussions of the invisible in nature,
"Merkwelt," and the problem of the ani-
l mind; "Das Neue Weltbild" reproduces
tropical aquarium in a series of splendid
rd-pictures, and contains two essays de-
ted to the nature of life and five to the con-
uction of a biological "Weltbild"; and
ally, under the heading "Spezielle Fragen"
find ideas on morphogenesis, Mendelism,
e origin of space, and a discussion of Paw-
w's work in which for the first time in the
viewer's experience trypsin is met with play-
g the rôle of the "Hauptsprengstoff für die
hwer verdaulichen Fette"! (p. 298).

Inasmuch as the arguments on which von
exküll bases his vitalistic teachings have been
scussed in an earlier review[1] and the pres-
t work contains nothing new in this respect,
may pass these over without further ado.
here are three matters, however, which seem
deserve fuller mention; the first has to do
ith the general purposes of the book, the sec-
d, with the temperamental backgrounds of
talism, whereas thirdly, we must consider a
method proposed for application in the field of animal behavior.

To begin with, then, von Uexküll does not aim his guns primarily at the body of trained unbelievers, but, profiting by the experience of the Darwinian period of open debate, attempts to recapture for vitalism the public opinion taken in the earlier period by mechanistic assault. Such victories are theoretically quite beside the mark, but no one can look into the history of things without forming the impression that public opinion played an important part in the advance of mechanistic doctrine.

For the execution of this turning movement von Uexküll appears well equipped. He is afire with enthusiasm, gifted with a pretty wit, and is master of a literary technic the like of which has not been seen in biological circles since the days of the great Darwinian apologists. Furthermore, temperamental qualifications of another sort do special service in the hands of our reformer, and this brings us to our second point.

As some men under the strains of life are driven to church, so others, impressed with the difficulties of biology, take to vitalism. The impelling embarrassments are partly objective and well known to all; others, however, are individual, and of these von Uexküll carries a heavy load. One who asserts, "Die Amöben bleiben zeitlebens ein strukturloses Protoplasmahäufchen" (p. 210) and who claims, "In ganz frühen Stadien . . . besitzt der Keim keine Struktur" (p. 270), must be constituted blind to some of the best things in modern research.

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[1] "Umwelt und Innenwelt der Tiere," SCIENCE,
S., *Vol. XXXI., pp. 303–305.*

addition to these and numerous other fic subjective results, our author also sees ie lay mind many dire effects of current iings. The world instructed by mechan- philosophers has lost the joy of life—"der ıenhimmel ist den meisten Menschen zu greulichen verworrenen Rechenmaschine rden, die ihnen einfach ekelhaft ist" 59); men spend their days in senseless ıeration; believe that all the invisibles in re are gases; accept a chemical morality ıot its mirrored image; and, by the grad- vorking inward of their algebraized sym- m, are ailing and dying at the heart.

w much of this tragedy is true to life how much a romantic adventure, is in the of recent events not so easy to determine. hard, however, to consider all this a nec- y consequence of mechanism, since Berk- implied long ago that thoroughgoing ıanism and idealism may dwell at peace ıe same mind. We are disposed to re- such lignifications of the heart and in- t, supposing them for the moment to have objective reality, not as the inevitable ts of a mechanism free from exaggera- pretension and carelessness, but rather e products of temperamental reactions to anism such as it unfortunately often is. f which is suggestive not only to those wonder why Germany, of all places, should a relatively favorable soil for modern biology, but to those also who ask her the vitalistic-mechanistic debate can osed.

ite apart from these matters, von Uex- treatment of the environment, and of elations of the organism thereto, has dis- ly practical interest for the experimen- . For Driesch the environment does not Neither index of the two volumes on 'Science and Philosophy of the Organ- contains the word, nor is any discussion e abode of life to be met with anywhere ıe seven hundred pages of dreary text. Uexküll, on the contrary, is all aglow for nvironment and its significance in the *retation of life.* Nor have these differ- *been without importance* for the two au- *nder comparison*; Driesch's reorganization of things biological has driven him out of the very field which should have proved more interesting than ever before; von Uexküll is continuing concrete observations and experiment and is pointing the way to further investigation with commendable fervor.

According to our writer, the environment of a living thing acquires special biological significance for us only when we discover and analyze those elements that actually act upon and with the organism in the normal give and take of daily existence. Such elements constitute the "Wirkungswelt." Further analysis differentiates out of the "Wirkungswelt" a "Merkwelt" which in the case of human beings, and perhaps wherever else it occurs, is specific for each individual.

These terms are practically self-explanatory. Air, for instance, is distinctly in our "Wirkungswelt," but may enter the "Merkwelt" under special circumstances. Now von Uexküll takes the position that students of behavior should limit themselves to a discovery by experiment of markworlds, and leave psychological considerations alone; brains and the objects of the external world can be experimented with, but of psychoses we can know one set only. The much exploited wonder-horses of Germany, the trained apes that open bolted doors, ring bells and order dinner, are monstrosities that have been forced to respond to the human order and not to normal constituents of either the horse- or monkey-world. Whether they have come to make these responses by trial and error, imitation or a system of rewards and punishments, is interesting only as a contribution to the art of unnatural history.

How the psychological difficulty is to be outflanked by this manœuver is not clear. The scallop's eye forms a retinal image like our own, yet the scallop "sees" only movements. To the specific forms, colors, sizes and the thousand other traits by which we distinguish one moving object from another this animal is blind. For the scallop a starfish, for us without taste or smell, has a pronounced odor indistinguishable from other chemical effects. Three marks in the following definite order (time-scheme as opposed to space-scheme) constitute a starfish in the scallop's "Merkwelt": movement, a general chemical mark, and a tactile stimulus. Given these in their orderly connection and the starfish is—"*wargenommen*"—that is, *perceived, observed, felt, taken care of, attended to,* or *availed of,* by the scallop. Clearly, until von Uexküll furnishes us with a system of notation by which the results of his experiments can be described without using words that suggest to every one the very thing upon which he has turned his back, discoveries concerning the markworld of the scallop are not likely to free us from the difficulties of an unanswerable question.

This does not mean that the method is inapplicable to cases in which the psychological question is respectable. On the contrary, James, in his essay "On a Certain Blindness in Human Beings," placed emphasis on the same spot fifteen years ago. In comparative psychology, and especially in experimental studies on human behavior, on the practise of education, and on the art of right living, numerous applications suggest themselves. If students adequately trained in the methods of science will seriously take up the experimental analysis of normal markworlds, we may expect significant results in a field which needs them perhaps more than any other among the biological substations.

Otto Glaser

University of Michigan

PAMPHLET

CAN A SINGLE SPERMATOZOÖN INITIATE DEVELOPMENT IN ARBACIA?

13

OTTO GLASER.

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CAN A SINGLE SPERMATOZOÖN INITIATE DEVELOPMENT IN ARBACIA?[1]

OTTO GLASER.

During the summer of 1913 while making the camera lucida tracings on which I have based my comparisons between the volumes of the unfertilized and fertilized ova of *Arbacia*,[2] it became necessary, in order to prevent rotation on the part of the eggs, and the consequent necessity of readjusting the focus, to employ very attenuated suspensions of sperm. The result of the highest dilutions used in these experiments, however, gave an unforeseen result since the appearance of the fertilization membranes was either very much delayed, or failed entirely to take place. This observation suggested the idea of a mass effect of the spermatozoa, and the possibility that this might play a rôle in normal fertilization.

At that time I had already made observations which had convinced me that the fertilization membrane in this egg is not formed de novo, but is preformed in the unfertilized egg, and simply rendered visible by changes occurring at the time of impregnation.[3]

The mechanism through which the fertilization membrane becomes visible will be dealt with in detail at another time; for the present it is sufficient to say that the absorption of water plays an important rôle. It occurred to me therefore that the prevention of this absorption and perhaps the prevention of fertilization itself might be possible even with the employment of more concentrated suspensions of sperm, if the eggs were first treated with Ca. As a matter of fact, it was either difficult or impossible to fertilize eggs so treated. The spermatozoa were active enough, but failed to enter, and fertilization membranes did not appear The following protocol is typical: In a small watch crystal, 4 volumes of fairly dense egg-suspension in sea-water+2 volumes n $CaCl_2$. After two minutes washed in sea-water.

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[1] From the Marine Biological Laboratory at Woods Hole, and the Zoölogical Laboratory of the University of Michigan.

[2] "The Change in Volume of *Arbacia* and *Asterias* Eggs at Fertilization," BIOLOGICAL BULLETIN, Vol. XXVI, pp. 84–91.

[3] "On Inducing Development in the Sea-Urchin (*Arbacia punctulata*), together with Considerations on the Initiatory Effect of Fertilization," *Science*, Vol. XXXVIII., pp. 446–450.

12.26 insemination moderate.
12.28 0 fertilization membrane.
12.30 1 " "
12.34 1 " "

Control normal. All eggs with fertilization membranes in 3–5 minutes after insemination. 100 per cent. cleavage. Hundreds of eggs examined in both control and experiment. In Ca-eggs very few divisions.

In connection with these experiments I noticed that insemination with great excesses of sperm frequently led to results at variance with the above, for fertilization membranes appeared about the majority of the eggs despite the use of Ca, and these eggs developed. This experience strengthened my belief, not only in the validity of the Ca-experiments, but also in the correctness of the original idea, namely that the number of spermatozoa that come into contact with the egg may make a difference.

Encouraged by this result, I diluted a sperm-suspension until only the faintest trace of opalescence remained. Several drops of this attenuated fluid were then drawn up into a medicine dropper of medium size and expelled quantitatively. If the dropper, which of course remained infected with sperm, was then used to agitate eggs in a small quantity of sea-water by carefully drawing the water in and expelling it several times, it was found that very soon a few spermatozoa had attached themselves to every egg. In an optical diameter, 4 to 5 sperm could easily be distinguished, but I awaited further changes in vain, despite the fact that the spermatozoa seemed to have reached the eggs, exhibited the usual amount of activity, and were potent in 100 per cent. of the cases when applied in larger quantities to eggs of the same lot. The following experiment is illustrative:

12.17 insemination with infected pipette.
12.18 0 fertilization membranes.
12.19 0 " "
12.20 1 " "
12.21 2 " "
12.22 3 " "
12.30 3 " "

Control normal; all eggs with fertilization membranes in 3 to 5 minutes. Experimental eggs examined at irregular intervals throughout the day, but no increase in the number of membranes.

Whether the appearance of a fertilization membrane, and impregnation itself will fail to take place in other eggs under similar conditions cannot be predicted, and is perhaps even improbable. With the eggs of *Arbacia punctulata* however I repeated these tests so often that I cannot doubt the correctness of my observations, and I therefore fail to understand Kite's[1] claim that he succeeded in calling forth a fertilization membrane in this egg by means of a single spermatozoön. I imagine that his method involved factors whose importance was unsuspected, since he says: "The real difficulty with this type of experiment is not the size of the spermatozoön, but the fact that when four or five are injected into the egg-jelly, they usually swim out and away from the egg. This necessitates the making of many injections in order to get a single spermatozoön to attach itself to the vitelline membrane and start the reaction." The "making of many injections" very likely involves touching the vitelline membrane an equal number of times, which recalls an experiment mentioned in my earlier paper[2] in which fertilization membranes were induced by surrounding the eggs with large numbers of minute infusoria. Observation indicated a continuous bombardment of the ova.

A quantitative relation between the rate of appearance of the membrane and the agencies, spermatozoa, normally calling it forth is really no more surprising than the efficacy of Ca as an inhibitor. Since now sea-water of sufficient hypotonicity will of itself call forth membranes[3] one may expect the exact reverse of the Ca-experiments if one immerses the eggs briefly in hypotonic solutions. Such ova, if not submerged too long so that the appearance of the membrane would have to be attributed to the hypotonic treatment itself, should be capable of fertilization by means of the sperm-infected medicine dropper. Actually under these circumstances fertilization with only 4 to 5 spermatozoa visible in the optical equator is possible in a considerable number of eggs.

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[1] G. L. Kite, "The Nature of the Fertilization Membrane of the Egg of the Sea Urchin (*Arbacia punctulata*)," *Science*, Vol. XXXVI., pp. 562–564.

[2] *Science, loc. cit.*

[3] In my preliminary communication (*Science. loc. cit.*) I considered the method of "inducing" a fertilization membrane in *Arbacia* by means of hypotonic sea-water new. Schücking however described this procedure in the year 1903. (*Arch. f. d. ges. Physiol.*, Vol. 97, p. 85.) The same method was used on *Arbacia* eggs by McClendon in 1910. (*American Journ. Physiol.*, p. 246.)

PROTOCOL.

In a watch crystal 3 volumes of sea-water+3 volumes of distilled. Added 1 volume of an egg-suspension in normal sea-water. At the instant when the first indications of membrane "initiation" were noticeable added 3 volumes of "double sea-water," *i. e.*, sea-water whose volume had been reduced one-half by boiling. By means of a sperm-infected pipette every egg was provided with 4 to 5 spermatozoa. In a series of microscopic fields the number of undivided eggs was later compared with the number that had divided. The results were:

Experiment I.		Experiment II.	
Undivided.	Divided.	Undivided.	Divided.
7	2	4	1
4	3	3	2
4	4	12	2
7	2	18	0
1	2	6	5
6	0	6	0
2	4	7	4
5	1	8	0
5	4	8	5
5	2	14	4
3	2	14	3
5	3	12	3
5	3	13	1
6	5	8	2
2	6	6	3
4	3	7	1
		13	2
		6	1
		6	3
		10	1
		8	2
Total...71	46	189	45
Per cent.61	39	81	19

Controls: Normal eggs+usual amount of sperm=100% Fertilization. Eggs treated as above+usual amount of sperm=100% Fertilization. This is in sharp contrast with the earlier experiments in which the operations were carried out at the same dilutions but without the brief fore-treatment with hypotonic sea-water. Results which harmonize with these but prove less satisfactory on account of injuries to the eggs can be gotten by the use of heat. In this case one might think of a parthenogenetic effect, but in *Arbacia* at least, it is not easy to confuse the usual parthenogenetic cleavage with normal two or four-cell stages.

It is very easy to misunderstand these experiments and to draw wrong conclusions. There is no more doubt in *Arbacia punctulata* than in any other form that a single spermatozoön is sufficient to carry out the biparental effect. Furthermore the experiments with dilute sperm do not in anyway enable us to prejudge what would happen in another egg under similar conditions nor do they warrant the inference that the initiation of development by a single sperm is impossible in *Arbacia* ova deprived of their superficial coverings. I feel very sure of this however: In *Arbacia* the appearance of the fertilization membrane after insemination is a sign that the egg investments have allowed the sperm to pass through. This passage has been possible because the coverings have changed. The change depends on a synchronous softening and absorption of water, the latter having consequences as the result of which the membrane becomes visible. Inasmuch as the becoming visible of the membrane is a reliable index of fertilization, and one of the consequences of fertilization is the division of the ovum, we may say that the initiation of development by a single spermatozoön in this case is impossible because a single sperm cannot effect those changes in the egg-coverings which will permit it to reach the protoplasmic surface film that lies beneath. The situation is exactly as though the entrance to a room were blocked by a barrier which a single man could not break down, although a group of ten might. Once broken down, any one of the men could cross the threshold, but for the opportunity of doing this, the services of the others would be needed. With this analogy in mind, the statement that a single spermatozoön cannot except possibly under special conditions, fertilize the normally invested egg of *Arbacia punctulata*, would appear to agree with the facts.

Zoölogical Laboratory,
University of Michigan,
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THE BASIS OF INDIVIDUALITY IN ORGANISMS

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THE BASIS OF INDIVIDUALITY IN ORGANISMS[1]

INTRODUCTORY

To enter upon the "higher criticism" of the concept individuality, is far beyond my powers. Even the humble attempt to think of it, in the organic realm, in what I conceive to be the simplest terms, offers difficulties most of which must be bequeathed in their entirety to future generations. Yet to point these out and to take a few soundings, unsatisfactory though they be, may not prove entirely futile even at this time.

For me, the basis of individuality in organisms is the mechanism by which living things, despite profound and constant change, keep themselves capable of identification. Some of the changes through which organisms pass are so radical that by common consent we treat them separately under the head of development, but since there is no evidence that living things become individuals at a particular point in their history, we may expect to find anywhere in the life-cycle the mechanism upon whose workings the possibility of identification rests. For obvious reasons the arrangements that make for constancy must occur in their least complicated form in the simplest of all the stages of development.

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[1] Read at a joint symposium of the American Society of Zoologists and Section F of the American Association for the Advancement of Science, Columbus, Ohio, December 30, 1915.

Fortunately, since it forces us at once to engage with fundamentals, the beginnings of development offer no refuge from our most insistent problem. We habitually identify a given organism at two more or less remote points of time, but no biologist limits himself to this relatively simple pursuit, since every living thing can be, at least partly, identified also with the better known portions of its ancestry. Indeed, these so-called genetic similarities are so striking and constant that one generation can be inferred from another with considerable precision.

If there is a substantial basis for the resemblances between parents and offspring, it must be the chromatin, for this is the only material capable of being contributed to each generation in essentially equivalent values by all the members of a given lineage. But if chromatin is responsible for the partial identifications possible between the individuals of two or more generations, we must also suspect that the specific recognition of a given individual at any of the numerous phases of his life is traceable to the same source.

THE SYNTHESIS OF CHROMATIN

Strictly speaking, "chromatin" is a morphological concept. Chemical analysis shows that it contains a conjugated phospho-protein provided with a nucleic acid group, the latter a complex of phosphoric acid and a nuclein base. During the so-called resting state of the cell, this material appears segregated in the nucleus.

We must attach to this substance a degree of specificity not less exact than the specificities we are seeking to explain. In this we have ample encouragement from cytologists and geneticists. But the question at once arises how chromatin can increase in quantity during more than one life cycle and yet lose none of its original characteristics. Brothers, who in the one-celled state derived from their mother the kind or arrangement of chromatin which in her father was associated with color-blindness, not only exhibit this defect in their own persons, but between the ages of 25 and 55 produce each some 169,692,750,000 examples of the same factor, all traceable to their own original endowment.

Compared with cytoplasm, the nucleus seems meager in the diversity of its chemical make-up. It is free from salts; it is devoid of fats and carbohydrates. Moreover, iron and phosphorus, easily demonstrable in the cytoplasm, are present in nuclei in forms difficult to detect and for that reason spoken of as masked or organic. These facts are not altered by doubting the localization of the iron in chromatin[2] or the accuracy of the tests for organic phosphorus.[3]

From the constancy of their occurrence we must conclude that both elements, as nuclear constituents, are essential. However, their absence in inorganic form, coupled with the general chemical poverty of the nucleus, indicates that simple raw materials for the synthesis of chromatin are excluded by the nuclear membrane (Macallum).

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[2] References in Aristides Kanitz, "Handbuch d. Biochemie," etc. Herausgegeben von Carl Oppenheimer, pp. 253–254, Bd. II., Teil 1.

[3] R. R. Bensley, *Biological Bulletin*, Vol. X., pp. 49–65.

This conclusion is out of harmony with prevalent interpretation. Yet no one need be misled. That nuclei are rendered conspicuous by staining, are scrupulously divided in cleavage and maturation, and combined with equal exactitude in fertilization, are all beside the point. Further, though no cell devoid of a certain proportion of nuclear material can live, it is no less true that a nucleus embarrassed by the loss of cytoplasm also fails to maintain itself. Chromatin, moreover, is present in the bacteria, but not in the form of a nucleus. Here its complete cytoplasmic synthesis is not open to doubt. We are ready enough to admit that the cytoplasm of nucleated cells can synthetize fats, carbohydrates, and proteins in general, including the most complicated compound forms. What real evidence have we that nucleoproteins constitute the sole exception?[4]

If we reckon with the synthetic powers of the cytoplasm as a possibility, we must next inquire how these can be influenced by the presence of a specific nucleus. That cytoplasmic response, in general, is dependent on the chemism of the cell, and that these activities are specifically and profoundly modified by changes in the variety of nuclear material present, are well-known facts shown nowhere more clearly than in the structural differentiations called forth in hybrids. These, especially, are important for us since the introduction of nuclei into foreign cytoplasm demonstrates most strikingly their ability to regulate syntheses so that more nuclei like themselves are produced. In what terms are we to conceive this regulation?

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[4] For a fuller discussion of the methods, evidence, and conclusions, see the articles by A. B. Macallum in Abderhalden, "Handb. d. Biochem. Arbeitmethoden," and in Ascher-Spiro "Ergebnisse d. Physiol.," VII.

The influence of a specific chromatin on cellular processes can be directly attributed to the samples which are known to leave the nucleus and come directly into the cytoplasmic reaction-sphere. But the details of their activity there remain obscure. Autocatalysis, suggested on quite inadequate grounds, is not necessarily excluded by the recent work of Conklin[6] and other effects are also thinkable. A fitness, chemical or physical in nature, between the liberated chromatin or its products, on the one hand, and certain of the reaction-products of cytoplasmic synthesis on the other, leading to the formation of different, or larger, non-reacting aggregates, would automatically increase the production of such substances, provided always the machinery necessary for their production is given at all. Very possibly the reciprocal relation suggested here is one of the keys to successful hybridization.

It is useless to hope for intellectual satisfaction in this matter at the present time. We can, however, assert with confidence that a cell is viable and assured of the possibility of offspring, essentially like itself, if it contains, at the beginning of its life-cycle, samples of all the various kinds of chromatin possessed by its immediate parent, and moreover, contains these in quantities sufficient to influence cytoplasmic syntheses so that they shall ultimately yield a chromosomal complex in which the original proportions among the several variants are quantitatively preserved.

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[6] E. G. Conklin, *Journal of Experimental Zoology*, Vol. 12, pp. 1–98.

THE SYNTHESIS OF CHROMOSOMES

If chromatin or its immediate forerunners are cytoplasmic in origin, how do they get into the nucleus? The impermeability of nuclear membranes for most constituents of the cell is probable; likewise, their permeability for nucleins, since these, even in the form of visible aggregates, seem to pass freely into the cytoplasm. If they can get through the membrane, going out, they can also get through, going in. The nucleus, therefore, is to be thought of as a kind of sanctuary into which certain proteins may enter, and, so long as they remain behind their wall, be free from the influence of other substances (Macallum).

These considerations are only an entering wedge. We infer a specific chromatin for each race, for every individual, and even for particular cells of the individual. More than this, in its intranuclear state, the chromatin is organized, in all likelihood, permanently, into chromosomes which exhibit symptoms, increasingly serious, of linear differentiation.[6]

If we admit the permeability of the nuclear membrane for chromatin or its immediate forerunners, we can with equal justification attribute the exact character of this membrane to the quantities and qualities of the substances enclosed. Specific permeabilities at once suggest themselves and so, by selective exclusion, any elements not true to one or the other of the types already present within the nucleus may, conceivably, be warded off (Macallum).

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[6] This evidence has been brought together conveniently by T. H. Morgan and others, in "The Mechanism of Mendelian Inheritance." The Macmillan Co., 1915.

Having admitted only specific elements to the nucleus, it becomes our duty to attach them to particular places in specific chromosomes. Here we are, necessarily, thrown on our resources in analogies.

Most suggestive is the behavior of optically active substances in various degrees of dispersion. The common Japanese camphor, dextro-rotatory in alcoholic solution, is also dextral in gaseous as well as solid form. A property therefore which in the highest and intermediate states of dispersal must be attributed to the configuration of individual molecules, is preserved in aggregates of these. This can only result from specific orientation.

Taken alone, this analogy is too simple. It may enable us to form some notion of the terms in which differentiation among the chromosomes is conceivable; but each chromosome, instead of being homogeneous, is, if we can trust ourselves, a system of heterogeneous complexes definitely arranged in space.

Our starting point may again be a relatively simple analog. The hexoses are also systems of heterogeneous complexes definitely arranged in space. While the actual form of the hexose molecule is unknown, the carbon atoms are distributed in a manner conceivable as a linear series in which aldehyde and ketone groups occupy the only positions possible.

Chromosomes, of course, are not large molecules, but aggregates of complexes of these. While the chemical forces determining the specific structure of the individual molecules may be precisely analogous to those which account for the nature of the hexose molecule, aggregation into linear series, in the case of the chromosomes, very likely involves elements not strictly molecular. There is one suggestion, however, that is bodily transferable to the situation presented by the chromosome, namely: factors, in the Mendelian sense, may occupy certain positions because these are the only loci possible.

In this connection, the temporary unions between enzymes and their specific substrates are especially interesting because they depend on the stereo-relations of large complexes of molecules. Conditions, generically similar, may play a determining rôle in the formation of more permanent unions even though these are not chemical. Stereometrically determinable fitness, degrees of fitness, or possibilities of fitness between various regions of persistent differentiated chromosomes and the newly synthetized elements by the lateral accretion or incorporation of which, these regions grow, enable us to visualize not only the periodic restoration of chromosomes to full size, but even the physical requirements for such phenomena as the single and double cross-over.

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THE DIVERSITY OF DESCENDANTS

We can hammer out, on the lines suggested, a provisional interpretation of that constancy in organisms which makes us call them individuals. But no two descendants of either compound or unicellular organisms are strictly alike. Each maintains an individuality of its own different from that of its immediate forerunners. This diversity must also be accounted for.

The differences between parents and offspring are adequately explained by the details of maturation. Why, however, do the units derived from the fertilized egg differ? This question is the inevitable consequence of our inability to consider more than one thing at a time. As yet we have neither reckoned with the differential distribution of cytoplasmic substances nor with the intimate history of the chromosomes during and after division.

Students of embryology are familiar with the distribution of "organ-forming" substances. These have been convincingly traced in a number of eggs (Conklin). The remarkable homologies found in the early development of molluscan and annelidan eggs of various types can be understood only as expressions of the accuracy with which these materials manœuver.

The visibility of an "organ-forming" substance is the merest accident. In the egg or cell from which an individual comes there may be and probably are materials whose accurate but uneven distribution during cleavage has not been noticed. Obviously there may be many occasions on which the cytoplasmic composition is changed during development.

Differential localization of itself indirectly increases the possibilities of further differentiation. With increase in the number of cells come purely physical and mechanical disturbances of equilibrium. In the readjustments that follow, changes of relation, themselves certain to influence the greatest variety of subsequent events, are inevitable. A crisis like gastrulation can not but affect, directly or indirectly, every cell in the system.

I am not forgetting the work of the Drieschian school of experimentalists. They have sinned abundantly in this field for the origin of two or four individuals from an egg whose blastomeres are separated at the appropriate moment by no means demonstrates a harmonious equipotential system. Harmonious it probably is, but equipotentiality is proved by meridional divisions only to those who consider them identical with equatorial or latitudinal cleavages. The production of viable organisms from blastulæ has been misinterpreted in the same way.

Differentiation may also be nuclear in origin. Not only are we unable to exclude the possibility of qualitative and quantitative disparities in ordinary mitosis, but we know positively that differences in nuclei may come about after division. We should recall the somatic cells of *Ascaris* and especially the differential growth of chromosomes.

As Conklin has pointed out[7] the chromatin mass does not necessarily double with each doubling in the number of cleavage cells, since growth is not shared pro-

[7] *Loc. cit.*

portionately by all the chromosomes. This fact, which very likely does not apply to the divisions of the sex cells, has been observed in the mitoses of early development, divisions which have been but little studied in detail. Such diminutions in the relative sizes of chromosomes may be accompanied by changes in the chromosomal balance and, through this, bring on changes of equilibrium among cytoplasmic processes. Some chromosomes may, in one respect or another, become ineffective, or in their altered circumstances may have effects qualitatively different from their earlier ones.

CONCLUSION

From the standpoint here adopted, differentiation is the expression of internal as well as external specificities. It is a cytoplasmic reaction and when it occurs denotes that something is not as it was before. Here as elsewhere, we do not deal with isolated events, but correlative changes with specific antecedents and specific consequences. This linkage of specified happenings persists through the entire life-cycle but in the adult, having few or relatively unimportant morphogenetic results, constitutes the basis for a physiology of maintenance.

In development as well as maintenance, that which constitutes our problem is a harmonic relation among all the processes whose net result makes possible the identification not only of an organism at any stage of life, but also of its ancestors. Such constancy, maintained despite the bewildering complexity and multiplicity of processes, is thinkable only in terms of the most rigid determinism.

The results of destroying portions of an embryo, the restoration of lost parts, heteromorphoses, the development of entire organisms from egg-fragments, grafting, the reorganization of an individual from its disjointed cells, and the fluidity of certain types of behavior, are in no sense counter arguments. All that these show is that the equilibria within which specificity is possible, have a certain range. When the eye-stalk of a crustacean regenerates, not an eye, which it does only under certain circumstances, but an antenna, the antenna is species-true, and when the stump grows an eye, which it does under circumstances of a different sort, but no less specific, the eye is not that of a man or an octopus.

If the developmental history of an individual yields a result from which his ancestry can be inferred, what other proof is needed for the accuracy of all the underlying processes? And what need have we who can think through our problems in materialistic terms for regulatory interference by metaphysical vapors? Far from making these things easier to understand, the table-rappings of the vitalist only withdraw attention from the one basis on which we can hope, at present, for a scientific account of the individual at all.

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THE THEORY OF AUTONOMOUS FOLDING IN EMBRYOGENESIS

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THE THEORY OF AUTONOMOUS FOLDING IN EMBRYOGENESIS[1]

THE experiments of Roux,[2] carried out on the embryonic chick, prove conclusively that the folding of a neural plate into a neural tube is not dependent, as His[3] had supposed, on the mechanical effect of one tissue upon another, but is autonomous. Self-differentiation in this instance is identical with self-folding. The question therefore arises: How can the neural plate fold itself?

Our reply must necessarily bear on all cases of autonomous folding, and reciprocally any one of them might serve as the basis for this analysis. The nervous system, however, is by far the largest, most easily studied, and, in addition, the most familiar of all the embryonic tissues in which self-folding occurs. Moreover, in its simpler forms, it indicates so clearly the direction in which an explanation of its autonomous transformations is to be sought, that for the present it seems best to limit the discussion to what may be justified as a type case.

For our immediate purposes, the neural plate of *Cryptobranchus allegheniensis* is especially suitable. Not only is it unusually large, as neural plates go, but wherever cell-boundaries are distinct, it is, without question, unicellular in thickness. The first problem to be solved is the rôle of cell-multiplication.

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[1] Read at the joint meeting of the American Society of Zoologists and Section F of the American Association for the Advancement of Science, in Columbus, December, 1915.

[2] "Die Entwicklungsmechanik," W. Engelmann, Heft 1, Leipzig, 1905.

[3] "Unsere Körperform, und das Physiologische Problem Ihrer Entstehung," F. C. W. Vogel, Leipzig, 1874.

In a neural plate in which the cells are irregular in position and dovetailed into one another as they are in crowded columnar epithelia, inequalities in the rate of division and protoplasmic synthesis at or near the two surfaces might lead to folding, but in the *Cryptobranchus* embryo, in which the plate is partly syncytial and in which the visible cell-walls are continuous from one surface to the other, and remain so during the entire period of folding, it is difficult to conceive how cell-multiplication could result in anything except uniform enlargement. The exclusion of this factor from participation in the process of involution, however, does not depend on mere argumentation, for comparison of the number of nuclei in comparable regions of the flat, half-folded, and completely folded plate, shows that the number of cells per section actually does not increase[4] (Table I.). Indeed in less simple material, such as the neural plate of the mammal, in which the number of cells does increase during folding, the restriction of the mitoses to the concave surface must, if effective

TABLE I

Number of Nuclei in Comparable Sections

Stage I, Flat	Stage II, Half-folded	Stage III, Folded
63	56	55
53	64	60
58	50	73
69	56	47
72	50	69
58	82	59
59	70	64
58	74	51
58	58	52
68	51	55
Ave. 62	61	59

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[4] For the validity of these comparisons see Glaser, *Anatomical Record*, Vol. 8, pp. 528–530.

at all, exert a force opposed to the forces that bring about the curvature. In this instance, therefore, a neural plate folds *in spite of* an increase in the number of its constituent cells.

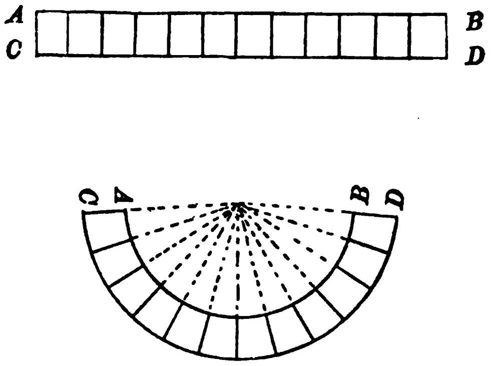

FIG. 1. Diagrammatic representation of a neural plate *A B C D,* conceived of, for the sake of simplicity, as entirely flat and made up of one layer of rectangular cells. The lower half of the figure shows the same plate symmetrically folded, its upper and under sides having become the outlines of two concentric circles. With the cells constant in number and position, the line *A B* is now necessarily shorter than the line *C D.*

The only remaining way in which a neural plate can fold itself is by a rearrangement of materials present at the beginning. In this connection the most patent fact, emphasized long ago by Rhumbler[5] and Conklin[6] in their studies of invaginate gastrulation, is a change in the shape of the cells whose sectional outlines alter from the rectangular form to that of a trapezium (Fig. 1). This geometrical transformation, which might be forced upon the cells from without, necessarily has the same result when autonomously produced, for it involves lengthening of one surface, short-

[5] "Zur Mechanik des Gastrulationsvorganges," *Arch. f. Entwicklungsmech.,* Bd. 14.

[6] "Mosaic Development in Ascidian Eggs," *Jour. Exp. Zool.,* Vol. 2, p. 163.

ening of the other, and a redistribution of the cell-contents. The extent of the latter, as indicated by the migration of nuclei from the side becoming concave to that becoming convex, is clearly shown for two sections in Fig. 2, and for a series, in Table II.

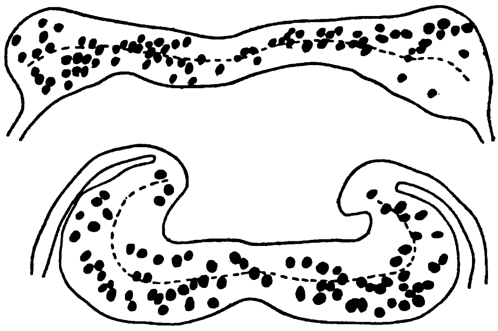

FIG. 2. Two sections through the embryonic nervous system of *Cryptobranchus alleghenienis*, showing the nuclear distribution in Stages I. and II. The sections are from the same series and regions as those dealt with in the tables but contain for Stage I., six, and for Stage II., one nucleus more than the maximal number recorded in Table I. In the unfolded plate there are in the present case, 78 nuclei, of which 47 are in the upper half above the dotted line, and 31 in the lower; in the half-folded plate, there are 75 nuclei, 21 in the upper zone, and 54 in the lower. Nuclei which happen to fall on the line separating the two zones are ascribed to the one into which the greater portion of their mass projects.

According to this, the distribution of the nuclei is not only completely reversed during folding, but the final relation between the number in what become the inner and outer zones, respectively, of the definitive tube, is as 2 to 1. Provided only that the nervous system is, by its structure and relations, incapable of indefinite expansion, these changes are all that are required to bring about the folding.

In my attempt to gain some insight into

TABLE II

Distribution of Nuclei in Upper and Lower and Inner and Outer Zones

Stage I, Flat		Stage II, Half-folded		Stage III, Folded	
Upper	Lower	Upper	Lower	Inner	Outer
32	31	31	25	15	40
32	21	22	42	15	45
34	24	16	34	21	52
55	14	27	29	13	34
39	33	18	32	22	47
38	20	27	55	16	43
31	28	33	37	26	38
33	25	29	45	13	38
37	21	21	37	20	32
44	24	19	32	20	35
Ave. 38	24	24	37	18	39

the manner in which these changes might be effected in the absence of coercion from without, I determined, at constant magnification, the areas of comparable sections during the process of involution. The marked relative increases shown in Table III. were found.[7]

TABLE III

Relative Areas of Comparable Sections

Stage I, Flat	Stage II, Half-folded	Stage III, Folded
9.4 cm.^{2}	13.7 cm.^{2}	17.5 cm.^{2}
8.1	11.3	19.5
10.6	11.4	19.5
9.9	10.1	20.6
9.1	10.1	20.5
9.6	10.9	18.1
9.1	11.5	19.0
8.2	12.0	18.5
8.3	10.2	18.9
9.9	10.1	18.8
Ave. 9.2	11.1	19.1

This increase in area indicates growth in volume, and can be the result only of enlargement on the part of the individual cells constituting the nervous system. The immediate problem is obvious.

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[7] For details concerning the distribution of this increase within the sections themselves, see Glaser, *loc. cit.*, pp. 530–533.

In Table IV. are given the water-content as well as the distribution of water in the larvæ of *Rana pipiens* and *Amblystoma punctatum,* four to five days after fertilization.

TABLE IV

Water Content and Distribution of Water in Embryos of Rana pipiens and Amblystoma punctatum Four to Five Days after Fertilization

Material	Fresh Weight, Grams	Dry Weight, Grams	Dry Substance, Per Cent.	Water, Per Cent.
R. pipiens:				
38 larvæ	0.1278	0.0557	43.6	56.4
50 larvæ	0.1718	0.0722	42.0	58.0
39 larvæ	0.1815	0.0730	40.2	59.8
41 larvæ	0.1788	0.0733	41.0	59.0
Average			**41.7**	**58.3**
24 yolk-sacs	0.0440	0.0204	46.5	53.5
31 yolk-sacs	0.0585	0.0264	45.1	54.9
Average			**45.8**	**54.2**
24 nervous systems	0.0464	0.0098	21.1	78.9
31 nervous systems	0.0714	0.0149	20.9	79.1
50 nervous systems	0.0916	0.0185	20.2	79.8
Average			**20.7**	**79.2**
A. punctatum:				
16 larvæ	0.0955	0.0399	41.8	58.2
15 larvæ	0.0992	0.0406	40.9	59.1
Average			**41.4**	**58.6**
125 nervous systems	0.3914	0.0785	19.9	80.1
52 nervous systems	0.1756	0.0400	22.8	77.2
15 nervous systems	0.0524	0.0106	20.2	79.8
69 nervous systems	0.2039	0.0363	17.8	82.2
Average			**20.2**	**79.8**

Since the period of differentiation under discussion has been completed at the stage of development considered in the table, and since this differentiation includes folding, and folding is associated with enlargement, it follows that the differential absorption of water by the nervous system probably took place during the process of involution. As the results show, the water-content rises to a point practically identical with the figure 80.5 per cent. given by Donaldson for the cord of the adult *R. pipiens.*[8]

But this absorption of water can only account for the enlargement of the nervous system, not at all for its folding.

To explain this in complete harmony with all the known facts, only one assumption is necessary. The neural plate is exposed to an external environment, whose constancy, within the limits under which normal development takes place at all, is very high. Laterally each cell of the plate is bounded by a chemical system fundamentally like itself. Disturbances of equilibrium on any one of these surfaces are relatively unlikely. However, on its under side, the plate is subjected to a constant change of conditions due to the multitude of processes going on within the rest of the embryo. To mention only one factor, there is a distinct increase in the acidity of the internal medium.

On this basis we may interpret the absorption of water as the result of a change in those surfaces of the absorbent cells which are exposed to the inconstant intra-embryonic environment. If this change involves a weakening of the face of the neural plate that becomes convex, the curvature that leads to the formation of a tube would be accounted for.[9]

Accordingly then, the absorption of water is not the cause of folding, but a symptom of that cause. If this interpretation is correct, the water content of the cells at any given level in the early stages of involution can not be uniform. In fact the theory demands that the marginal cells of the neural plate, the first, it will be recalled, to undergo a change of shape, shall have a higher water-content than the cells in the middle of the plate which only assume the wedge-shape during the last stages of involution.

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[8] Donaldson, Henry H., "Further Observations on the Nervous System of the American Leopard Frog, etc.," *Jour. Comp. Neurol.,* Vol. 20. Also earlier papers.

[9] For the relation between this view and the Rhumbler Surface-Tension Hypothesis, as well as for a criticism of the latter, see Glaser, *loc. cit.,* pp. 536–548.

For the decision of this crucial question, no direct method is as yet available. However, it is possible to secure evidence indirectly which seems to me convincing.

If the eggs of the starfish are placed in hypotonic sea-water, and given an opportunity to absorb more water than they normally contain, they at once increase in volume, and their nuclei, easy to deal with on account of their spherical shape, also enlarge. The facts on which this statement is based are given in Table V.

TABLE V

Asterias Eggs in Various Concentrations of Sea-water

No. of Eggs	Conc. Sea Water	Conc. Dist. Water	Diam. Eggs	Diam. Nuclei
18	100%	0%	142μ	.68μ
	66	34	170	.82
23	100	0	138	.66
	75	25	144	.70
47	100	0	152	.66
	60	40	188	.82
49	100	0	154	.66
	60	40	170	.80

Before applying this information to the problem in hand, I had first of all to determine whether these facts held for the nervous system, and especially whether measurable differences could be demonstrated in those regions known to have contained during life, different proportions of water.[10]

[10] That the embryonic brain has a higher water content than the cord is indicated by the figures which I published in SCIENCE, N. S., Vol. XXXIX.,

TABLE VI

Relative Water Contents of Embryonic Cords and Brains

	Embryonic Cords		Embryonic Brains
Amblystoma	125	>	125 by 2.2 per cent.
Rana	139	>	135 by 1.9 per cent.
Rana	192	>	188 by 2.3 per cent.

Relative Sizes of Nuclei

Nervous System of *Cryptobranchus Embryos*

Stage I			Stage II			Stage III		
Cord	Brain	Ratio	Cord	Brain	Ratio	Cord	Brain	Ratio
109	109	1:1.2	126	114	1:1.1	125	113	1:1.3
119	114	1:1.2	107	108	1:1.1	143	123	1:1.3
120	125	1:1.1	127	129	1:1.2	133	113	1:1.3
121	119	1:1.1				115	131	1:1.3

Control 36-hour Chick

End of Cord	Forebrain	Ratio
110	124	1:1.4

Relative Sizes of Nuclei in Center and at Edges of Neural Plate in Cryptobranchus during Folding

Number and Positions of Nuclei

Central	Lateral	Ratio
110	115	1:1.2
112	120	1:1.2
112	111	1:1.1
135	122	1:1.2

In both *Amblystoma punctatum* and *Rana pipiens* (Table VI.), a comparison of the anterior and posterior ends of the embryonic nervous systems, indicates a higher water-content in the larval brain than in the cord. Since these results are consistent, and, in sense, agree with corresponding differences found by Donaldson (*loc. cit.*) for the adult nervous system of *Rana pipiens,* I feel fairly certain of the essential correctness of my values, and infer, therefore, that the embryonic brain, like that of the adult, also has a water-content higher than that of the cord at the same age.

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pp. 730–731, in 1914. The evidence there presented was meager and, unfortunately, I overlooked some arithmetical errors. Recalculation has made no essential difference in the results, however, and further evidence now shows them to have been essentially correct.

If this is indeed correct, and, moreover, if nuclear volume varies with the water-content of the cell, and, furthermore, if fixation does not destroy or completely reverse the volumetric relations, one would expect the nuclei in the anterior end of an embryonic nervous system to be larger than those in the posterior.

In *Cryptobranchus* embryos such comparisons are easily made. The nuclei are large so that errors, inevitably committed in determining their volumes, are relatively small. Certain precautions however are essential. Thus nuclei in various stages of mitosis must obviously be excluded. Also, since the resting nucleus is ovoid in shape, it is necessary to consider only those similarly oriented with reference to the plane of section. Absolute volumes are, of course, not practicable, nor are they requisite. All that the theory demands is that the average size of the nuclear sections in the regions which had the higher water-content shall be greater than those in the regions in which the water-content was lower. Tracings of some 2,800 nuclei whose outlines on paper were cut out with scissors and weighed under uniform conditions of atmospheric moisture, give results remarkable for their uniformity.

The absolute regularity of the ratios based on *Cryptobranchus,* and on the control observation on the thirty-six hour chick, convinced me that nuclear size, even in preserved materials, can be utilized as an index of original water-content. If now, the absorption of water is itself an index to the surface alteration to which I attribute the change in shape undergone by the cells during involution, then the nuclei of the lateral curling edges in any given section should on the average be larger than those in the, as yet, unfolded center. This, as indicated in the last division of Table VI., is true for *Cryptobranchus.*

Since this expectation has been fulfilled, I feel that the problems involved in the autonomous folding of the nervous system, and by implication, also involved in such other autonomous foldings as that of the entodermal plate in typical invaginate gastrulation, have begun to merge with the physical-chemistry of the tissues concerned, and the conditions to which their constituent cells are subjected at various periods of development.

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THE HYDROLYSIS OF HIGHER FATS IN EGG-SECRETION.

OTTO GLASER

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THE HYDROLYSIS OF HIGHER FATS IN EGG-SECRETION.

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THE HYDROLYSIS OF HIGHER FATS IN EGG-SECRETION.

OTTO GLASER.

I.

In four recent publications ('15, '18, '21, and '22) Richards, Miss Woodward, and I have dealt with the enzymic properties of *Arbacia* egg-secretion. It was shown, first of all, that radiation affects these secretions as though enzymes were present; next, there was precipitated a body, lipolysin, which, like unmodified exudate, was subsequently shown to accelerate the hydrolysis of ethyl butyrate. Finally, in my last paper ('22), I reported the synthesis of butyric ester.

On the basis of these results, we may assume that lipolysis plays a rôle in the initiation of development. The position is greatly strengthened by the fact that normal eggs may be completely sterilized if their secretions are removed by short exposure to charcoal ('21). Nevertheless the view that initiatory changes are somehow linked with the activities of a lipolytic enzyme requires further evidence. So far the results with ethyl butyrate are the clearest of all. However, if these were due, as they might be, to a relatively specific esterase, the fact, though interesting from other points of view, would be difficult to fit into a theory of fertilization. In order that lipolysis may find a place as part of the mechanism of initiation, it must first be shown that the enzymes present in egg-secretion actually affect the hydrolysis of higher fats.

II.

Preliminary tests were made with olive oil. This was carefully neutralized with dilute NaOH and subsequently extracted with fresh water, and later with ether ('09). The oil was then ready for use.

If egg-secretion influences the hydrolysis of olive oil, the effect, under the proper conditions, might be rendered apparent through changes localized at the oil-exudate interface. Here important disturbances should take place, although mere volumetric changes are not likely to be reliable. The diffusion of the glycerine set free in hydrolysis would, of course, result in a decrease in the volume of the oil drops. On the other hand, the production of oleic acid with its great affinity for oxygen and its capacity for breaking down into lower fatty acids might easily balance, or even overbalance, the loss resulting from the solubility of the glycerine in egg-water. Conceivably, then, the drops might decrease in volume, increase in volume, or even remain constant.

Nevertheless, if hydrolysis takes place, a lowering of the surface tension at the oil-exudate interface is to be expected, and, regardless of volumetric considerations, should bring about a concentration at the phase-boundary of substances either in solution or in suspension within the oil. If such substances happen to be insoluble in egg-secretion, one may likewise expect their precipitation immediately about the periphery of a drop from which glycerine and possibly the oxidation products of oleic acid, are diffusing outward.

I used two indicators; in some tests Sudan III. was dissolved in the olive oil, in others pulverized charcoal was suspended. A sharp phase-boundary, permitting a clear focus under the microscope, was secured by placing small discs of the oil on the surface of the exudate. The observations were checked by comparisons made on sea-water and distilled, as well as by the use of oil without indicators. Additional controls were carried on with solutions of pancreatin and the commercial Holadin.

With discs of plain olive oil on sea-water, there is some marginal effect. This is indicated by the rather rapid development of a moderately irregular outline. On distilled water the discs maintain their smooth circular form almost perfectly during the first few hours of an experiment. The differences in these two cases are no doubt traceable to the sea salts.

If egg-secretion is used in place of sea-water, the oil discs in the course of an hour take on a form suggestive of a circular saw whose projecting teeth are in a state of active disintegration. Small particles can be seen breaking off in large numbers until the originally sharp outline of the disc becomes quite obscured. Similar effects were noticed on sea-water, but they develop less rapidly and, within the time limit of these experiments, never equaled the effects gotten with egg-secretion.

With Sudan III., in the early stages of an experiment, peripheral elimination and precipitation of the dye on either sea-water or distilled is questionable. On egg-secretion, however, there is no doubt. The outlines of the discs become exceedingly irregular; there is marked surface concentration of the stain, and very minute particles of it are precipitated densely about the periphery. After 12 hours the oil discs are quite gone. In place of them one finds irregular islands, stained with Sudan III. After 24 hours a rancid odor is noticeable.

With pulverized charcoal exactly comparable results can be gotten, only the surface changes are even more striking. After 24 hours the islands on egg-water can be distinguished macroscopically from their controls, partly because of their greater irregularity, and partly because they have become vesiculated.

If the differences noted are due to the activities of a lipolytic ferment, then solutions known to contain fat-splitting enzymes should reproduce these differences. Attempts were made with two products, the one labeled Pancreatin and of unknown origin, the other a Parke-Davis preparation with the trade name Holadin. Both of these, especially in experiments in which charcoal was used, gave very striking effects and, after 24 hours, the irregular vesiculated islands could be distinguished even macroscopically from their controls.

III.

In these first experiments I made no attempt to control bacterial action. Whatever may be true after six, twelve, or twenty-four hours, the earliest noticeable differences between the oil discs on exudate and on sea-water became apparent so quickly that bacterial digestion seems unlikely. This fact, therefore, warrants a more careful examination of the fat-splitting properties of the secretion.

I now prepared exudate as free as possible from bacterial contamination. The females were thoroughly washed in running fresh water and carefully dried. Only shed eggs were used and these in filtered sea-water. The secretion itself was filtered several times through Chardin paper and the remaining infection controlled by the addition of chloroform. KCN was not employed because it checks the action of certain lipases.

With exudates of this type I shook up varying proportions of either olive oil or whale oil and at stated intervals attempted to titrate the systems with NaOH N/40 back to the specific turning points of such indicators as phenolphthalein, neutral red, or litmus. On account of the buffer effects and saponifications due to the salts present in both secretion and sea-water, such titrations, even if otherwise reliable, can not disclose the total acidity.

But there are other difficulties. Digests of the type here under consideration are almost proverbially mean. In addition, these particular systems in egg-secretion, in the course of an hour or two, underwent serious physical changes. At the beginning of an experiment, oil and secretion, after shaking, would separate promptly, but after standing for the stated times at 20° C. the separation was much less complete. After four hours even moderate shaking imparted to the systems a stability almost jelly-like.

Under these circumstances, even with the addition of neutral alcohol ('03), titration gave highly variable results, and hence nothing in the nature of a curve showing the course of the reaction can be plotted. However, if we take the most reliable titrations—*i.e.*, those made upon volumes measured before the physical changes previously referred to had taken place—certain very definite comparisons are possible.

Series *A*	40 c.c. secretion 10 c.c. olive oil	40 c.c. sea-water 10 c.c. olive oil
After 45 hrs.	10 c.c.=2.7 c.c. NaOH *N*/40	10 c.c.=1.9 c.c. NaOH *N*/40
Series *B*	150 c.c. secretion 25 c.c. olive oil	150 c.c. sea-water 25 c.c. olive oil
After 30 min.	10 c.c.=1.5 c.c. NaOH *N*/40	10 c.c.= .4 c.c. NaOH *N*/40
Series *C*	90 c.c. secretion 25 c.c. olive oil	90 c.c. sea-water 25 c.c. olive oil
After 30 min.	10 c.c.=1.8 c.c. NaOH *N*/40	10 c.c.=1.3 c.c. NaOH *N*/40

These values indicate the number of c.c. of NaOH N/40 necessary to return the several systems to P_{H_8}, using phenolphthalein as the indicator. The relatively high acidity found in the controls is a disturbing factor, but unavoidable, since oleic acid is never entirely absent in a "neutral" oil; is, moreover, constantly being produced; and, as constantly oxidized to dioxystearic and, very likely, other lower fatty acids. The differences between the digests and the controls, however, remain significant.

IV.

By far the most fruitful observation was made, not on the digests themselves, but on the litmus which in certain cases was used as the indicator. As compared with the controls in sea-water and oil, and in exudate without oil, neutral litmus, when shaken up in mixtures of secretion and either whale oil or olive, instantly becomes pinker. After two hours the pink is distinctly intensified, and after twelve, such digests stand out sharply from their controls.

Within twenty-four hours all color, including the pink, disappears in the oil-secretion digests. If more litmus is now added, it again turns pink, and, in the course of time, fades out completely. The controls, on the other hand, even after forty-eight hours, still appear "neutral," with perhaps only the faintest leaning toward pink.

It is plain that the litmus is not functioning here as a direct indicator of acidity. Very likely the change to pink and the final complete decolorization are the first and last visible steps in a process of reduction. But why, we may ask, the difference between the secretion digests with neutral oil and the controls? If the explanation is to be found in the superior reducing powers of the oil-secretion systems, then these must be producing a substance capable of binding oxygen. Moreover, they must be producing this material at a rate far in excess of the rate in the controls.

Now, if the oils in the presence of secretion are undergoing ordinary lipolytic cleavage, one of the reaction products must be oleic acid, and this, as is well known, absorbs oxygen with great ease. Is it possible, then, to attribute the decolorization of the litmus to the reducing powers of oleic acid? We can only do this if we can first show that oleic acid is present in higher concentration in the digests than in the controls; and, if we succeed in proving this, we shall incidentally also furnish the proof that the only source of oleic acid—the neutral oil—is undergoing accelerated hydrolysis in the presence of egg-secretion.

V.

With this idea in mind, I prepared a special set of tubes, using two volumes of egg-secretion, one volume of either neutral olive or whale oil, and one volume of a neutral litmus solution. The tubes were "chloroformed" as before, then stoppered and shaken.

The secretion used in these tests was made with every precaution possible, and I kept a microscopic check on each tube. In a few instances I could find no organisms whatever either at the beginning or at the end of the period of digestion; in other cases the number was too slight to account for the results, since control tubes, deliberately infected to the point of cloudiness, required forty-eight hours to produce a barely noticeable reduction of the litmus, whereas in the oil-secretion digests the reduction always began instantaneously.

Tubes without chloroform became turbid within forty-eight hours and developed the unmistakable rancid odor. In the tubes with bacterial growth inhibited no rancid odor could be noticed on account of the masking effect of the chloroform. This necessitated some other indicator for the presence of oleic acid.

The test finally chosen was based on a statement of Hammarsten's ('12), and depends on the fact that oleic acid in the presence of cane sugar and sulfuric acid develops a purple color. The usual method was to place a drop of the digest on a slide, to mix with this a drop of saturated solution of cane sugar, and to add from 2 to 5 drops of concentrated sulfuric acid.

Care must be taken not to add the sulfuric too quickly, or to use too much, for if the sugar breaks down suddenly, the carbon set free obscures the reaction in the oleic acid. With these precautions, however, the test is delicate and very reliable. The change in the oleic-acid globules can easily be followed under the microscope. It begins as a slight discoloration, succeeded by pink—rose—and finally a deep purple. At 20° C. the reaction is slow with freshly formed oleic acid, but proceeds more rapidly after the oleic has been exposed for some time to the air.

By this method it was possible to demonstrate the presence of oleic acid in the digests in which chloroform had masked the rancid odor. Moreover, the concentration of oleic acid, as shown by the number, size, distribution, and depth of color of the purple globules, left no doubt that hydrolysis was proceeding at a faster rate in secretion than in sea-water. In the controls an occasional globule did turn purple, but this was to be expected from our inability to prepare, or, for that matter, even to keep, a fatty oil absolutely neutral.

VI.

It may be taken as demonstrated, then, that *Arbacia* egg-secretion has the power to hydrolyze higher fats. Since neither whale oil, olive oil, nor ethyl butyrate occur in sea-urchin eggs, we must conclude that the lipase present is non-specific.

The question now remains whether the lipolysin first isolated by Miss Woodward also affects the hydrolysis of higher fats.

To determine this, I repeated the experiments described in the preceding section, using, in place of egg-secretion, sea-water solutions of the lipolysin precipitate. The concentrations employed were, roughly, 25 milligrams in 10 c.c. of solvent. To report the results in detail would be a mere reiteration of the experiments with egg-secretion. In every case the evidence for hydrolysis was positive, and leads to the conclusion that the precipitated lipolysin is, or contains, that enzyme which in unmodified egg-water is responsible for the hydrolysis of the higher fats.

AMHERST COLLEGE,
February 4, 1922.

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TEMPERATURE AND FORWARD MOVEMENT OF PARAMECIUM.

20

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TEMPERATURE AND FORWARD MOVEMENT OF PARAMECIUM.*

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I.

Paramecium is extremely sensitive to slight changes in its environment and in consequence has been used, since the days of Spallanzani, as a delicate biological indicator. Yet, despite the three thousand or more papers dealing specifically with this organism, quantitative studies of its relations to the environment have been exceedingly rare and very limited in scope. So far as concerns temperature, for example, we find Pütter (1903),[1] in a curious state of confusion, denying that the production of mechanical energy by this animal is in any way proportional to an increase in heat intensity. Since then, Kanitz (1915)[2] has found in Khainsky's (1911)[3] results on *Paramecium caudatum*, support for his own earlier and even more irregular experiments with the contractile vacuoles of other Infusoria, while Woodruff and Baitsell (1911–12) determined $Q_{10} = 2.84$ or 2.70, for the fission rate over an interval of only 4°. With respect to temperature, certainly, the advantages of sensitivity in Paramecium remain largely unexplored.

The analysis here proposed deals with the effect of temperature on forward motion. This is perhaps the most common act which the animal performs and is, therefore, at all times available for experiment. The purpose of the study does not include an attempt to illuminate the mechanical arrangements that propel the organism;

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* Owing to Dr. Glaser's absence in Europe, proof of this paper has been read by the Editors.

[1] Pütter (1903), p. 88.

[2] Kanitz (1915), p. 62.

[3] Khainsky (1911), p. 32.

of energy. A series of underlying reactions of this sort necessarily has a definite sequence, and the most elementary consideration suffices to show that the rate at which a system of linked processes can proceed as a unit depends absolutely on the component with the slowest rate. In its relations to temperature, therefore, forward translation should reflect the corresponding properties characteristic of the controlling reaction. Accordingly, the experimental

TABLE I.

Average Time Required by Paramecium to Swim a Unit Distance at Different Temperatures.

Temperature.	No. of observations.	Time.		
		Observed, average.	Calculated. $\mu = 16{,}000$	Calculated. $\mu = 8{,}000$
°C.		sec.	sec.	sec.
6.6	7	2.64	2.65	
7.0	7	2.58	2.60	
9.9	6	1.98	1.98	
10.7	18	1.89	1.81	
10.8	10	1.80	1.80	
14.8	10	1.22	1.22	1.19
15.0	10	1.17	1.18	1.18
16.0	26	1.10	1.08	1.12
17.5	50	1.04	0.94	1.04
18.0	10	0.91	0.90	1.01
18.5	24	0.89	0.86	0.99
18.7	10	1.09	0.84	0.98
19.0	40	0.95		0.97
19.4	20	0.91		0.95
21.2	10	0.81		0.87
21.5	15	0.71		0.85
22.0	36	0.83		0.83
23.0	32	0.80		0.80
25.0	10	0.74		0.73
25.4	11	0.73		0.72
25.5	15	0.77		0.72
27.0	14	0.65		0.67
27.3	12	0.63		0.65
29.0	40	0.69		0.62
30.0	100	0.56		0.59
39.0	30	0.43		0.41
40.0	7	0.40		0.39

points should be calculable by means of the Arrhenius[6] (1915) formula of 1889 which describes the changes in the rate of acceleration of a chemical process at different temperatures and which has been employed with such success by Hecht (1919, 1918–19) in his studies on the influence of temperature on photic sensitivity in *Mya*.

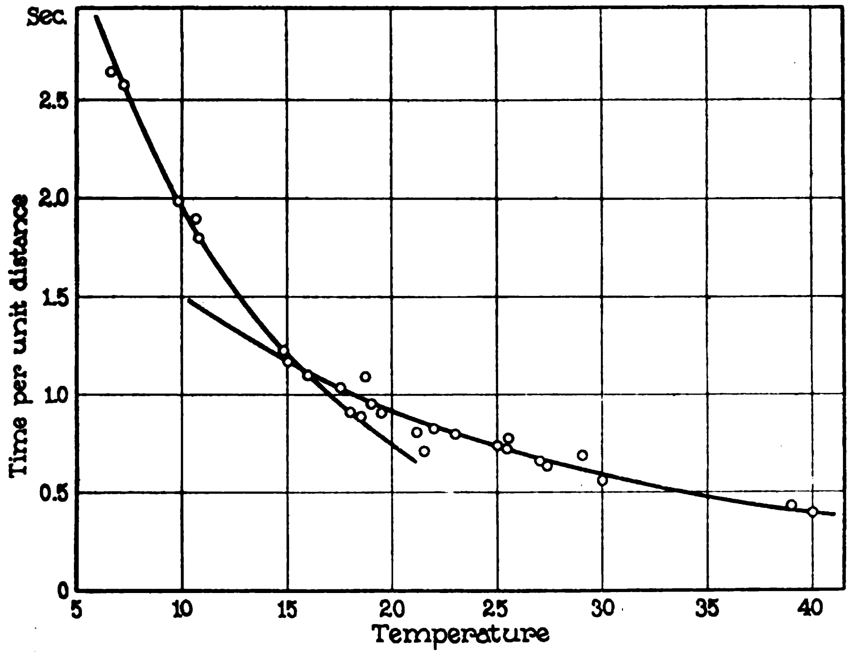

FIG. 1. The Arrhenius equation applied to the relation between temperature and time in the forward translation of *Paramecium*. For the left-hand curve, $\mu = 16{,}000$; for the right-hand, $\mu = 8{,}000$.

In the Arrhenius expression, the ratio between the velocity constants K_1 and K_0 of a chemical reaction taking place first at one, then at another temperature is related logarithmically on the Naperian base e to the gas constant, the absolute temperatures, and to a constant μ which characterizes a particular reaction. As formulated,

$$\frac{K_1}{K_0} = e^{\frac{\mu}{2}\left(\frac{T_1 - T_0}{T_1 T_0}\right)} \tag{1}$$

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[6] Arrhenius (1915), p. 49.

From the values in Table I velocity constants cannot be calculated. However, for K_1 and K_o respectively, we can substitute the reciprocals of the times, $\frac{1}{t_1}$ and $\frac{1}{t_o}$, required to perform the same amount of work. The temperature characteristic, μ, can then be easily determined by transformation and a convenient change from the Naperian to the Briggsian base.

Calculated in this manner for the entire range from about 6–40°, μ, far from being constant, shows a marked decline. This, however, is not proof of a theoretical misconception at the outset, for as Crozier and Pilz (1923–24)[6] have pointed out, over a temperature range of this extent the effect is not necessarily described by a single curve, even if smooth. On the contrary two curves, each with its own characteristic value for μ, may be required for adequate description. In the present case, and within the limits of experimental error, a curve with μ = 16,000 fits closely from 6.6° to about 16°, whereas a second, with μ = 8,000 simulates the data remarkably well from about 15–40°. How closely within their respective ranges the values given by the theoretical curves coincide with those experimentally found, is shown strikingly in the plot and also in the last three columns of the table.

IV.

The divergence of the two theoretical curves between 15° and 19° is significant. Within this range of temperature several of the points might be attached perhaps equally well to either curve; nevertheless, in this region originates a change of direction which above 20° is unmistakable. What does this mean? On the principle of a controlling rate which we have assumed as the basis for our analysis, the problem finds a simple solution. The speed of the entire system of underlying reactions is increased by a rise in temperature, yet it does not follow that all components of the system have the same acceleration. A specific reaction—the slowest one prior to a certain change in temperature—may be replaced by another which even if originally faster, nevertheless with rising temperature drops into the last place.

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[6] Crozier and Pilz (1923–24), p. 720.

The idea involved here is not new. As Cohen-Stuart (1912)[7] found it necessary to emphasize, van't Hoff himself expressly stated that in chemical systems Q_{10} remains constant only at certain temperatures. However, the existence and possible significance of a definite break in biological data, particularly at points where the processes under consideration begin to occur at supernormal temperatures, were clearly brought to light only recently by Crozier and Federighi (1923–24) (see also Crozier and Pilz, 1923–24[6]). In certain arthropods, the process underlying the state of reflex immobility (so-called "death-feigning") at low temperatures has a μ value of 24,000, while from 16–30°, $\mu = 9{,}200$. For other processes (Crozier, 1924–25, *a*, *b*, and *c*) corresponding extremes are 16,400 and 11,200. Hecht (1918–19) reported for the latent period of *Mya*, in the lower ranges, a value of 19,000, and slightly over 11,000 at 31°. With these instances forward translation in *Paramecium* aligns itself easily, for aside from the fact that in *Mya* μ declines gradually, all these cases have all these points in common: high values of μ go with low temperatures and *vice versa;* the high values fall within one general order of magnitude, and the low ones within another; and when breaks occur, these are found abruptly where the temperatures exceed those at which the animals or tissues normally operate.

V.

For our analysis the physical meaning of μ has important consequences. Historically this constant traces back to 1872 when Guldberg (Arrhenius (1912)[8]) found the rate of change in the logarithm of the dissociation pressure directly proportional to the heat of dissociation, and inversely, to the gas constant and to the square of the absolute temperature. The expression of this was applied by van't Hoff in 1885 to chemical velocities, and in 1889 appeared the Arrhenius simplification. The descent of μ in the theoretical line, then, is perfectly clear.[9] In its latest guise it bears the original relations to the absolute temperature and the gas constant. As used by Arrhenius, therefore, μ fundamentally stands for, and in the most favorable data is proportional to, and perhaps identical with, a heat of dissociation or reaction.

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[7] Cohen-Stuart (1912), p. 1162.

[8] Arrhenius (1912), p. 83.

[9] In his reference to the empirical equation of Arrhenius, Rice (1923, p. 2808) does not distinguish sufficiently between a formula which is truly empirical and one whose use, even if discovered by arbitrary methods, nevertheless depends on constants thoroughly grounded in physical theory.

Quite recently, F. O. Rice (1923) has brought forward considerations which if fully substantiated must greatly increase the utility of μ. Arrhenius explained the effect of temperature on the hydrolysis of cane-sugar by HCl in watery solution by postulating that the sugar was present in two forms, the active and the inactive, in mass action equilibrium, and that at equilibrium the concentration of the active form was very low. Rice assumes that in acid catalysis the H ion rather than the sugar exists in two forms, the hydrated ion and the unhydrated, and that the latter is the active form and is present in low concentration because of the great affinity between the H ion and water. An exactly comparable assumption is applied also to hydroxyl ion catalysis. In this way it appears possible to explain why μ has practically identical values for so many reactions superficially different. Far from being a meaningless accident, the fact becomes rationally clear if we follow Rice's suggestion and focus our attention on the chemical mechanism by which transformations are brought about rather than as heretofore on the primary substances transformed (Crozier, 1924–25, *a*, *c*). If this point of view is correct, chemical reactions must group themselves into a relatively small number of classes, and each class must possess a characteristic μ, because in each class of reactions the mechanism involved is the same.

In his analysis of the data on the mammalian auricle, echinoderm eggs, the breathing rhythm of insects, the reduction of hemoglobin, and respiration in nervous tissue and in bacterial processes where the velocities measured are probably those of oxidation, or at least, are functions of respiration, Crozier (1924–25, *a* and *b*; Crozier and Federighi, 1923–24) has found consistently values in the neighborhood of 16,000. The quite independent discovery of $\mu = 16{,}000$ for forward translation in *Paramecium* suggests, therefore, an oxidation as the controlling reaction for the lower ranges.

In the present state of our knowledge an attempt at the class identification of the reaction whose rate of acceleration is measured by $\mu = 8{,}000$ is perhaps even more hazardous. In order of magnitude, this value might not fall outside the class of hydrolyses. It is true that Rice (1923) finds 10,950 from Reicher's data on the hydrolysis of ethyl acetate by sodium hydroxide and from Wilsdon's studies on the hydration rates of acetic, propionic, and camphoric anhydrides, an average of $\mu = 11{,}100$. On the other hand, in a list of μ values for a variety of processes, Arrhenius[10] includes 7,540 for the saponification of cottonseed oil by the lipase of castor beans, while for the tryptic cleavage of casein, $\mu = 7{,}400$. Until we have much more definite information in this field, commitments regarding specific reactions are hardly warranted.

VI.

In conclusion we may ask whether it is possible to conceive a chemical reaction system capable of accounting for the distribution of the data and for the apparent transfer of velocity control from one process to another in different temperature ranges.

The observations themselves and their analysis do not limit us particularly to any specific mechanism. The simplest conception applicable to the controlling reactions is a catenary series $O \rightarrow A \rightarrow E$ in which an original source of supply O is changed into the available form A, whose destruction to the end-products E, yields the energy resulting in translation. If $O \rightarrow A$ has a μ value of 8,000 while for $A \rightarrow E$, $\mu = 16{,}000$, the depletion of A at higher temperatures would proceed at a faster rate than its replenishment, and hence the increase in the rate of the process as a whole would become less as time went on.

As an explanation, this scheme cannot be supported by the sort of evidence that would rule out every other possibility. We may in fact be dealing, at the higher temperatures, with the increasing effect of some process that interferes with the flow of $A \rightarrow E$ by diverting a fraction of A. Indeed, the data apparently will bear an interpretation similar to the one which led Hecht (1918-19) to postulate a heat inactivation in connection with the photic response of *Mya*. Such a mechanism, however, is not so simple, and the data for *Paramecium* do not enable us to decide between these two possibilities.

[10] Arrhenius (1915), p. 54.

While the less complicated idea accounts for the facts and hence is preferable as a first approximation, the mere dealing with velocity controls only, lends a deceptive simplicity to the suggested mechanism. As a matter of fact, if we consider *A* in the catenary series a reservoir in the main supplied from *O*, and consider *E* as partly oxidized and partly reversible to *O*, Meyerhof's conception of carbohydrate metabolism in muscle becomes quite applicable (Shaffer, 1923). *Paramecium* undoubtedly also oxidizes carbohydrates, and unless the metabolism is radically different, the process runs in a cycle and is composed of two main steps. In muscle, according to Meyerhof, glycogen is hydrolyzed to glucose and lactic acid during the anoxidative phase, whereas the oxidative results in the oxidation of both these products as well as in the reconversion of three-quarters of the lactic acid to glycogen. If we attach the lower μ value (8,000) to the synthesis of glycogen from lactic acid—an assignment reasonable in view of the large quantities of water produced during this phase—Meyerhof's conception should give a fairly well substantiated reaction system capable of the velocity controls demanded for the present case in *Paramecium*. Moreover, Meyerhot's circular scheme accounts well not only for the transfer of control but allows for the fact that the data can be interpreted as involving at the higher ranges a deficit of *A*. If we assume for normal temperatures a quantitative adjustment such that the prevailing rate $O \rightarrow A$ keeps the concentration of *A* constant at the prevailing rate of oxidation and of the reaction $A \rightarrow E$, and then assign $\mu = 16{,}000$ to the oxidative phase, temperatures lower than normal would result in no change of control, but possibly in an accumulation of *A*. At higher ranges on the contrary, owing to the depletion of *A*, the rate of the system as a whole would come to depend more and more on the synthetic transformation of *E* to *O*, the forerunner of *A*, and thus, concretely, if *Paramecium* were a muscle, on the conversion of the lactic acid back to glycogen. Obviously such details must await further investigation. The available data merely bring the analysis of forward translation of *Paramecium* into the sphere of physical-chemical methods and conceptions.

SUMMARY.

1. The rate of forward movement in *Paramecium* as affected by changes in temperature can be described accurately in terms of the Arrhenius equation.

$$\frac{K_1}{K_0} = e^{\frac{\mu}{2}\left(\frac{T_1 - T_0}{T_1 T_0}\right)} \qquad (1)$$

2. For the range from 6–15°, μ = 16,000; from 16–40°, μ = 8,000. These values fall within the limits characteristic for chemical processes.

3. On the principle of velocity control by the slowest rate, it is assumed that in *Paramecium* at temperatures above normal, control passes from one underlying reaction to another.

4. The views expressed by Rice, the recent results of Crozier, and certain μ values given by Arrhenius all suggest that μ = 16,000 may represent an oxidation, and μ = 8,000 either a modified oxidation or an hydrolysis.

5. For the system of controls, the catenary series $O \rightarrow A \rightarrow E$ with the lower μ value attached to the precursor reaction is adequate. We may also assume a cyclical system analogous to Meyerhof's conception of carbohydrate metabolism in muscle. In this case it is necessary to assign μ = 16,000 to the oxidation of A and E and μ = 8,000 to the synthesis $E \rightarrow O$. This model also accounts for the fact that the data might be interpreted as involving, apparently, a depletion of A at the higher temperature.

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TEMPERATURE AND THE MECHANISM OF LOCOMOTION IN PARAMECIUM.

21

Charles

By OTTO GLASER.

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TEMPERATURE AND THE MECHANISM OF LOCOMOTION IN PARAMECIUM.

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(Accepted for publication, May 7, 1925.)

I.

The effect of temperature on the velocity of forward movement in *Paramecium* is described accurately by the formula of Arrhenius, the values for μ being 8,000 in the upper ranges and 16,000 in the lower (Glaser, 1924–25). This result rests on the ordinary averages derived from the behavior of numerous individuals all belonging to a single pure line. At each temperature, however, averages mask the limits of variability; and these limits are highly significant in the precise study of thermal increments (Crozier and Federighi, 1924–25; 1925). For this reason a further analysis of the data on *Paramecium* is desirable. Moreover, for the temperatures considered by Crozier and Federighi, linear translation in *Oscillatoria* filaments, as well as the heart beat of *Bombyx* larvæ, yield thermal increments constant at 9,240 and 12,200 respectively. It is additionally interesting, therefore, to consider also a case in which averages indicate a sharp break in the neighborhood of 15°.

II.

The material available for this analysis consists of 709 records in which the time required for *Paramecium* to swim a constant distance at different temperatures was measured with a stop-watch.

Inasmuch as the details of these experiments have been fully described (Glaser, 1924–25) it is necessary to consider only the methods employed in the present handling of the data. The latter are influenced by three conditions each of which can be a source of artefacts. First, the number of observations is not constant over the entire range, and hence this number at each temperature must be describe the points as closely as possible rather than that they should in all respects conform to statistical convention. As a matter of fact the most striking qualities of normal probability curves are clearly present; there is an evident approach to bilateral symmetry and inflection occurs on ordinates of standard deviation from the mean. However, the calculated "theoretical" maxima diverge somewhat from the maximal percentage frequencies actually found; yet until a "theoretical" maximum in biological statistics can be accredited in dynamic terms, it appears safer to assume that the greatest relative credibility attaches to the points representing the greatest number of observations.

TABLE I.

Temperature.	No. of observations.	Minimum.		Maximum.		Mode.		Mean.
		Frequency.	Log time.	Frequency.	Log time.	Frequency.	Log time.	Log time.
		per cent		*per cent*		*per cent*		
10.4	60	1.7	1.52	1.7	1.02	29.9	1.22	1.26
15.0	47	2.1	1.32	2.1	.92	34.1	1.07	1.08
17.5	60	3.4	1.14	3.4	.89	36.6	1.03	1.03
18.4	64	1.6	1.18	1.6	.83	30.4	.98	1.00
19.1	70	4.2	1.08	1.4	.83	35.0	.98	.94
22.0	46	8.7	1.01	6.5	.76	30.4	.91	.91
23.0	41	2.4	1.06	7.2	.76	34.0	.91	.89
25.5	100	1.0	1.02 1.12	10.0	.72	35.0	.87	.85
27.0	72	5.5	.93	6.9	.68	35.9	.83	.81
29.0	40	17.5	.92	2.5	.62	35.0	.82	.83
30.0	109	8.2	.87	5.5	.62	30.9	.72	.74

It is desirable to check the validity of these curves in another way. If the groupings on which they rest do not distort the data, then log velocities deduced from the log time classes should behave in a predictable manner. Plotting their values according to Crozier's method (1924–25) against reciprocals of absolute temperature, should result, above 15°, in four straight lines, one for the means and one each for maxima, minima, and modes. These curves should be parallel and should have increments of 8,000. In an ideal case these values would fall between the lines describing maxima and minima respectively and these might be equidistant from the one describing the means. Below

15° the corresponding curves should give $\mu = 16{,}000$, though the distance between the maximal and minimal lines might easily exceed or be smaller than it is in the higher ranges. How closely these conditions are fulfilled is shown in Table I and Fig. 2.

It seems reasonable then to accept the frequency polygons as they stand. Without insisting that their value exceeds that of a useful approximation, we may conclude on the basis of spread and height that in the region above 15°, and again very possibly also below, the variability in the time required to swim a unit distance shows no tendency to rise or fall, but remains essentially constant.

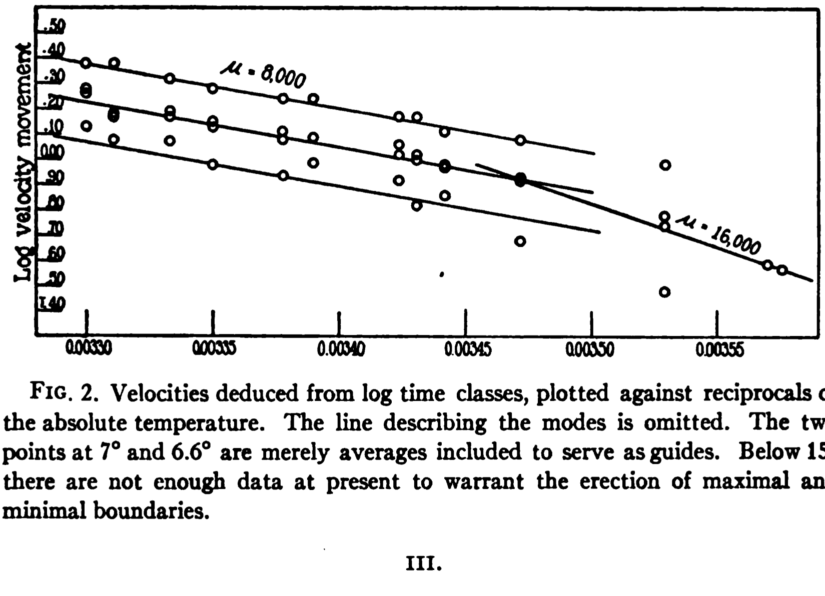

FIG. 2. Velocities deduced from log time classes, plotted against reciprocals of the absolute temperature. The line describing the modes is omitted. The two points at 7° and 6.6° are merely averages included to serve as guides. Below 15° there are not enough data at present to warrant the erection of maximal and minimal boundaries.

III.

Constancy above 15°, substantiated as it is, suggests an essential homogeneity of the material. Apparently a rise from the "critical" point to 30° does not seriously change the character of the system which controls the speed. This fact, for which evidence is now available, was merely an assumption necessary for the theoretical explanations offered in my previous paper. It is not surprising then that the facts of variability should easily harmonize with the earlier analysis based on averages alone.

The underlying mechanism assumed to account for this behavior of

Paramecium in relation to temperature is a chemical reaction system controlled by a catenary series in which O, a source of original supply, is changed into an available form A, which on destruction to the end-product E, yields the energy that results in movement. It was suggested that for $O \rightarrow A$ μ has a value of 8,000, and for $A \rightarrow E$, 16,000. Under these conditions a depletion of A is likely and would explain the decreased rate of acceleration at the higher temperatures. The case presented by the frequency polygons is in principle the same. We can suppose, with Loeb and Chamberlain (1915) and with Crozier and Federighi (1924–25), that differences in the velocity of different individuals and of the same individual at different times can be attributed to differences in the effective concentration of certain catalysts. This assumption merely transfers the control of $O \rightarrow A$ and of $A \rightarrow E$ to catalytic agents either inevitably present during the passage of $O \rightarrow A \rightarrow E$ or to catalysts in origin independent of the major reactants. If now the formation of such active catalysts has a temperature coefficient equal to that of the biological process under consideration—in the present instance, forward movement in *Paramecium*—it follows that the slowest individuals and the fastest, as groups, must have rates of acceleration equal to that of their mean, to which the extreme speeds should be related as constant fractions.

Above 15° this condition is closely approached by the polygons and by the curves in Fig. 2. Below 15° there is also some evidence indicating constancy in their relations, but whether the constants are the same must remain questionable until sufficient data are available for the lower range. Inasmuch as control at the lower temperatures by assumption rests with another catalyst, it might well be that its limits of variability in concentration are different from those of the catalytic agent controlling the reaction system above the "critical" point (*cf.* figures in Crozier and Stier, 1924–25).

The system of control then can be represented as

$$\begin{array}{ccc} O \xrightarrow{\hspace{2em}} & A \xrightarrow{\hspace{2em}} & E \\ \mu_c = 8{,}000 & \mu_c = 16{,}000 & \end{array}$$

(μ_c pertaining to catalyst) which though slightly more complicated than $O \rightarrow A \rightarrow E$ involves no change in the underlying conception. Moreover, analysis in terms of controlling catalysts in the long run may result in great simplifications since the reactions and reacting substances in living things are innumerable, whereas the number of catalysts involved may possibly prove to be quite small.

SUMMARY.

1. Frequency polygons in which the number of observations at each temperature is reduced to a percentage basis while the time factor is represented on a logarithmic scale, indicate that the time required by *Paramecium* to swim a unit distance at different temperatures varies within definite limits which are constant above 15°. Below 15° the range of variability very possibly is not the same, though probably likewise constant.

2. Logarithmic velocities deduced from mean, maximal, minimal, and modal time classes, when plotted against reciprocals of the absolute temperature, fall respectively on straight lines. These lines are parallel and give μ values of 8,000 above 15° and probably 16,000 below.

3. This implies that the mechanism of locomotion in *Paramecium* remains essentially unaltered by a rise in temperature from 15° to 30°, and probably remains in a similar sense constant from 6° to 15°.

4. The theoretical interpretation of this result is possible in terms of a catenary series $O \rightarrow A \rightarrow E$ in which the passages $O \rightarrow A$ and $A \rightarrow E$ are controlled by two catalysts differing respectively in concentration in different individuals and perhaps at different times in the same individual, but depending for their effective concentration on reactions having temperature coefficients identical with those which at each temperature characterize the biological process under consideration.

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TEMPERATURE AND HEART RATE IN PTEROTRACHEA AND TIEDEMANNIA.

22

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TEMPERATURE AND HEART RATE IN PTEROTRACHEA AND TIEDEMANNIA.

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(Accepted for publication, October 23, 1925.)

I.

The attempt to give form and substance to various possibilities suggested by a study of thermal increments at present calls for an increase in the available data. It is necessary to analyze a greater array of biological processes in a greater variety of forms. Since the fauna at Naples affords abundant opportunities for investigations of this character, a number of organisms fairly common there were explored and, for the purposes in mind, the heart rates of the large heteropod *Pterotrachea coronata* and of the pteropod *Tiedemannia neapolitana* proved to be, in several respects, almost ideal. Aside from the great convenience of being completely transparent, both animals are very sensitive and permeable to heat, and their hearts are large enough to be seen clearly without a lens, under practically all circumstances. At intermediate temperatures pulsation in *Pterotrachea* in unit time is approximately twice as fast as in *Tiedemannia* where ten beats require slightly over 0.4 of a minute between 14° and 15°. Moreover, there are significant differences and resemblances between these two forms,—a fact which has acquired additional interest since Crozier and Stier (1924–25, *a*), have reported one temperature characteristic for the heart rate of *Limax* ($\mu = 16{,}300$), and another for *Anodonta* ($\mu = 11{,}200$). With these values at hand, it is now possible to compare and interpret the increments for two other molluscs widely different in systematic affinities, in mode of life, and in physical consistency.

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* The observations and experiments here recorded were made during the winter of 1924–25 while occupying the table supported at Naples by the American Association for the Advancement of Science. I take this opportunity to express my deep gratitude to both the Association and the Administration of the Stazione Zoologica. It is perhaps worth noting that under the leadership of Dr. R. Dohrn the conditions necessary for effective scientific work in the Naples laboratory are rapidly approaching the ideals of today.

II.

The observations on *Pterotrachea* were made after placing each animal in a separate aquarium, open to the air and containing about 1200 cc. of sea water. These vessels were stationed in a glass tank in which the level of the water was kept above that in the individual containers. To reach the upper ranges of temperature, definite amounts of water were removed from both the tanks and the aquaria and carefully replaced by equal quantities at higher temperatures. In this way both the levels and the differences of level were held sufficiently constant. The lower temperature limits were reached by allowing small flasks filled with ice, and corked, to float on the surface of the water in the containers, and by adding cold water or ice to the outer water jacket. In this case also, precautions were taken to maintain the original levels.

Final readings were begun at the lowest limits and on increasing the temperature 5 minutes were usually sufficient to establish the new equilibrium. In bringing this about, the uniform distribution of heat in the inner chambers was greatly facilitated by the gentle rhythm of the dorsal "fin" of the mollusc and moderate undulating movements on the part of the entire animal. *Pterotrachea* is almost never completely quiescent. The thermometers, like the animals, were always entirely submerged and after a given temperature had been maintained within 0.1–0.2° for 5 minutes, the time required for ten complete cycles of the heart was determined with a stop-watch. In the earlier observations this process was repeated five times for each temperature, but later only three groups of ten beats each were counted. The results derived in this way from eight individuals and covering a range, collectively, from about 4–27°, are plotted in mass in Fig. 1 according to Crozier's method (1924–1925, *a*) and the familiar Arrhenius equation (1915).

It is at once apparent that individuals must differ decidedly in the actual rate of the heart beat at any given temperature; also, that within definite limits, both vertical and lateral on the plot, the observations may be represented by a straight line. The thermal increment that best describes the points between $1/T = 0.00340$ and $1/T = 0.00350$, is 11,200. Above and below these regions other conditions plainly hold.

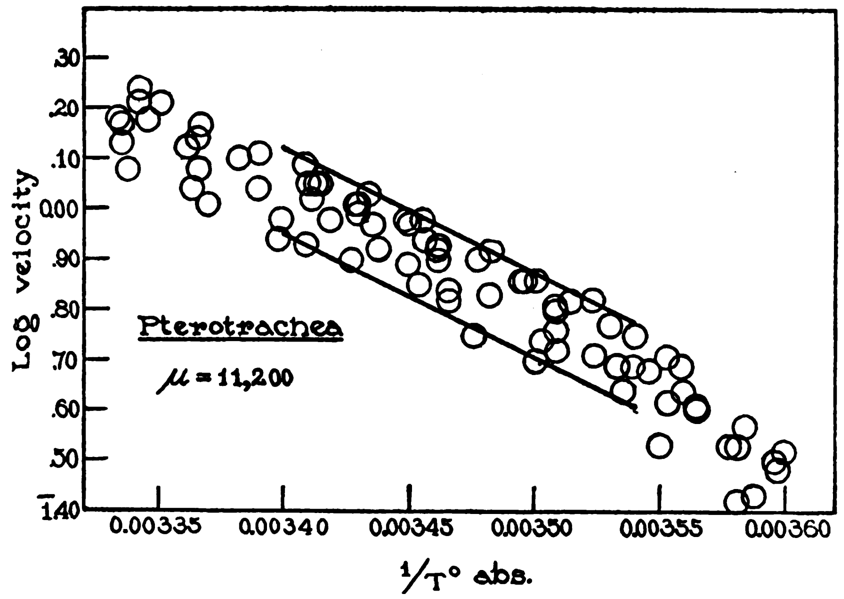

FIG. 1. Velocity of heart beat in *Pterotrachea coronata;* mass plot. Some of the points represent fifty beats but most of them, thirty. The slope is in accordance with the Arrhenius equation,

$$ln \frac{K_1}{K_0} = \frac{\mu}{2}\left(\frac{1}{T_0} - \frac{1}{T_1}\right)$$

For intermediate temperatures, $\mu = 11{,}200$.

It is hoped that the presentation of all the data will avoid the criticism that might be levelled at selected cases. These, in view of certain difficulties reported by Rywosch (1905), would not be altogether adequate. Indeed this author considered the heart of *Pterotrachea* essentially lawless because at any given temperature the rate may be quite different in different individuals. Rywosch also notes that sudden changes such as plunging an animal from 15° to 40.5° and returning it after 1 minute to 16.5° may stop the heart at once or dissociate auricular from ventricular rhythm. Such drastic methods of course produce results quite different from moderate physical violence or small but abrupt changes in temperature. Indeed, these alone are enough to induce some of the effects demonstrated by Rywosch; they may even initiate disharmonies of rhythm that become noticeable in the rate, or bring the heart to a standstill, not immediately, but at some quite different and totally innocent point on the temperature scale. Manipulative and experimental ineptitudes like these are perhaps useful in suggesting methods by which the heart might be controlled, but unfortunately they may also prove highly misleading.[1]

TABLE I.

Pterotrachea Heart.

Animal.	μ (intermediate).
1	11,200
2	11,900 (twice).
3	11,100
4	11,000
5	11,000
6	11,500
7	10,800
8	11,100
Average............................	11,200

From our general plot we should expect a reasonable uniformity among the increments deduced for separate individuals. This, as the list in Table I indicates, is true.

In selecting cases for individual presentation, it seemed best to choose the two that are farthest away from the average and the one that happens to exhibit the increment of 11,200. The data for these individuals are plotted in Fig. 2, and illustrate the precision with which the heart accelerates at intermediate temperatures. They also show, though not to greatest advantage, the terminal changes at extreme temperatures.

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[1] Rywosch (*loc. cit.*, p. 361) writes: "Es lässt sich keine allgemeine Formel aufstellen in welchem Verhältniss etwa die Zunahme der Zahl der Pulse zur Erhöhung der Temperatur steht; ganz allgemein scheint es nur zu sein dass bei höheren Temperaturen auf einen Wärmegrad mehr Pulse kommen als bei niedrigeren."

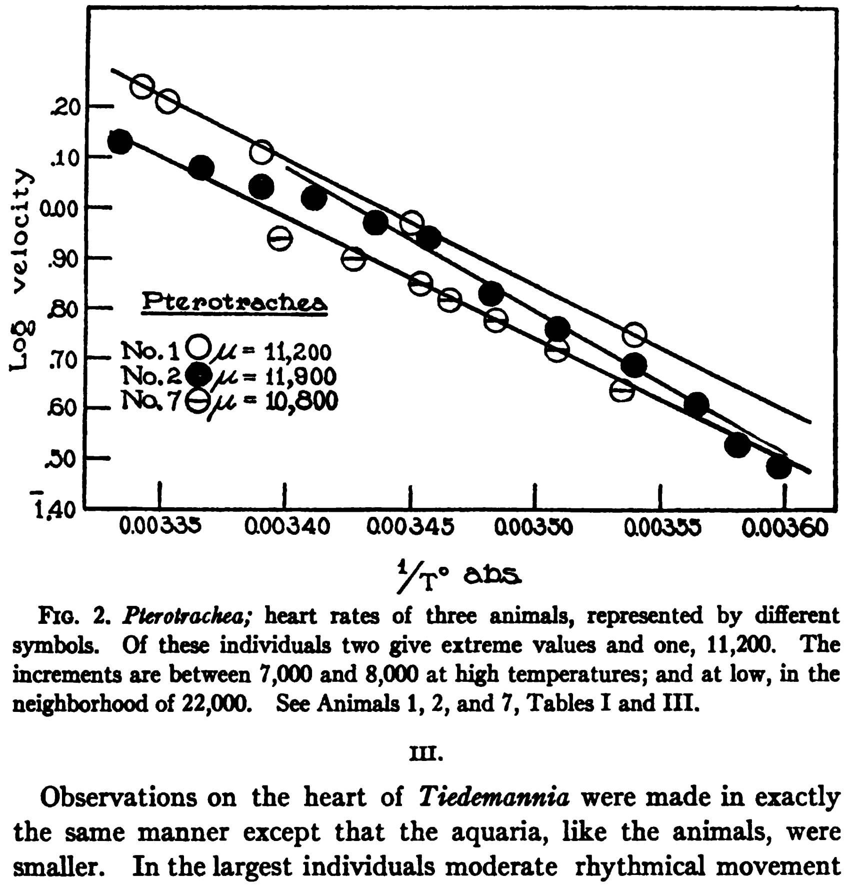

FIG. 2. *Pterotrachea;* heart rates of three animals, represented by different symbols. Of these individuals two give extreme values and one, 11,200. The increments are between 7,000 and 8,000 at high temperatures; and at low, in the neighborhood of 22,000. See Animals 1, 2, and 7, Tables I and III.

III.

Observations on the heart of *Tiedemannia* were made in exactly the same manner except that the aquaria, like the animals, were smaller. In the largest individuals moderate rhythmical movement of the "wings" facilitates the distribution of heat and does not obscure the view of the heart; in the smaller animals, however, it is often necessary to wait for periods of complete quiescence. In a general presentation of the data in a single plot, *Tiedemannia* in the middle ranges of these experiments exhibits an increment of 16,200.[2]

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[2] Four out of eleven individuals exhibit an increment of 14,500±. The explanation cannot be found in manipulative irregularities committed in the course of experimentation. The points on which this increment is based could hardly demonstrate its reality with greater clearness. The matter will be discussed later.

These curves are in every way comparable with the results for *Pterotrachea.*

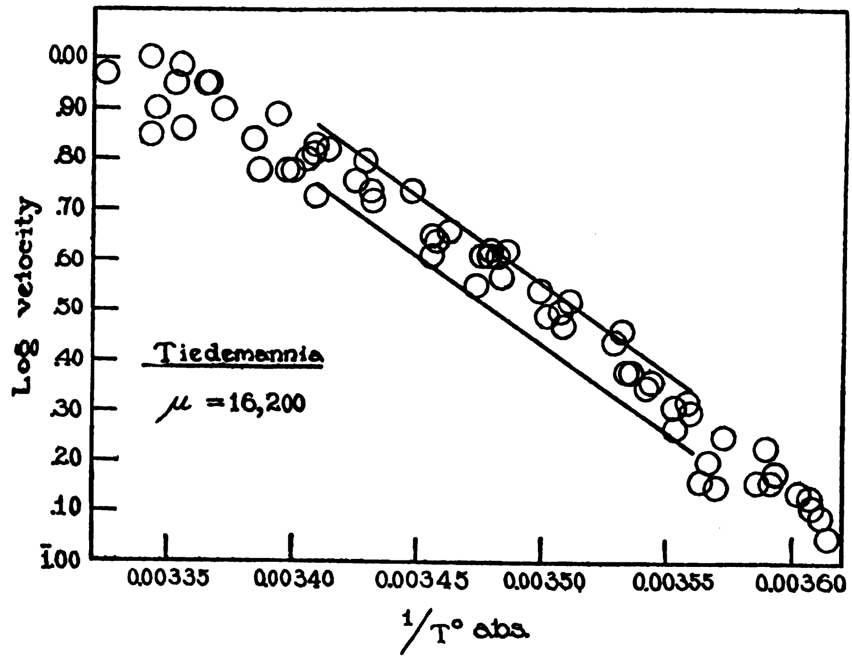

FIG. 3. *Tiedemannia neapolitana;* mass plot. At intermediate temperatures, μ = 16,200.

TABLE II.

Tiedemannia Heart.

Animal.	μ (intermediate).
1	16,000
2	16,300
3	16,200
5	16,100
6	16,500
9	16,100
10	16,100
Average............................	16,200

IV.

Until further studies on the upper and lower limits of the temperature field are available, the results for these regions must be considered as essentially an orientation. Necessarily, observation in these distal ranges is greatly restricted. At both high and low temperatures certain individuals show no change of increment; whereas in others, even with the exercise of great care, the heart beat becomes irregular, intermittent, or stops altogether. Near the upper limits, an intermission may be followed, significantly, by great and sudden variability. It is very difficult at times to be certain what the resulting points imply. Again, the onset of a shift in μ values appears to be influenced by the rate at which the temperature has been changed. Excluding the instances in which μ is either incalculable or unchanged,

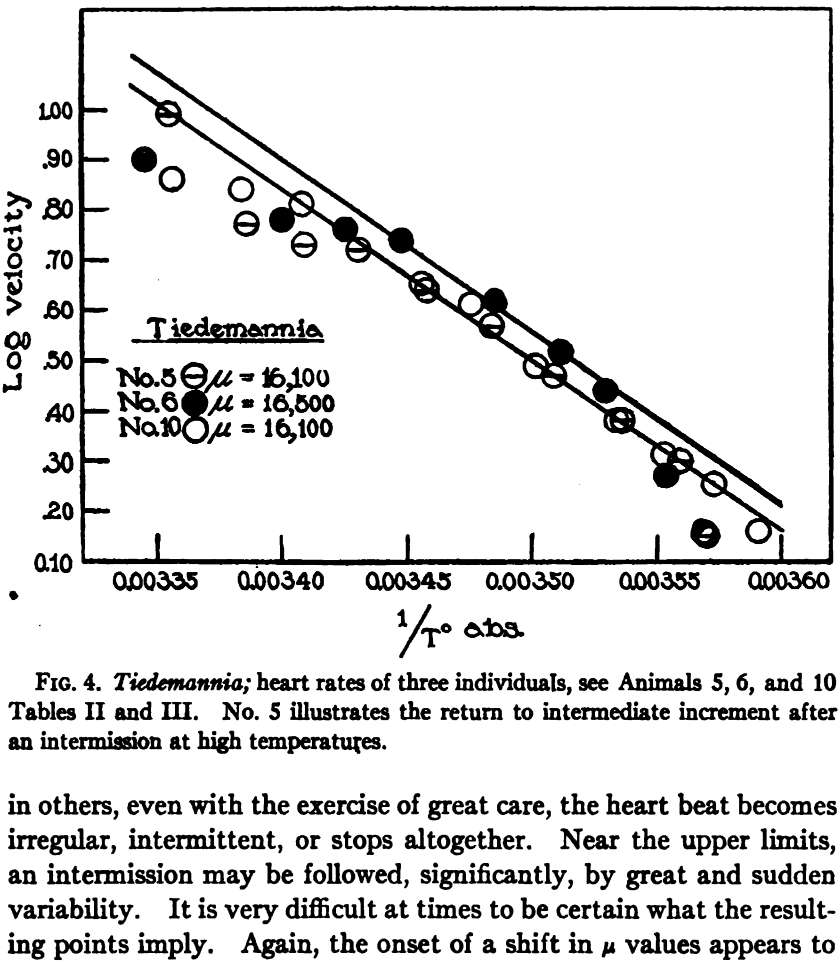

FIG. 4. *Tiedemannia;* heart rates of three individuals, see Animals 5, 6, and 10 Tables II and III. No. 5 illustrates the return to intermediate increment after an intermission at high temperatures.

it becomes relatively easy to rate the terminal increments as to their general order of magnitude with fair precision. As indicated in Table III, the values for μ at the extremes are sufficiently constant and different to suggest for *Pterotrachea* and *Tiedemannia* hearts the existence of apparently not less than two distinct terminal controls.

TABLE III.

Terminal Values for μ.

Pterotrachea No.	Temperatures.	
	High.	Low.
2	7,200	20,600
2	6,900	28,000
3	8,300	22,500
4	7,800	20,500
5	7,500	21,800
6	—	17,400
7	8,400	—
8	4,900	—
Average................	7,300	22,000
*Tiedemannia** No.		
1	7,000	—
3	9,200	20,100
4	5,900	21,700
5	7,000	—
6	7,200	27,800
7	8,900	20,900
8	7,400	26,400
9	7,200	—
10	5,900	—
Average................	7,400	23,000

* This table includes the terminal data for the individuals with intermediate increments of 14,500±. These it will be observed are indistinguishable from those where the intermediate $\mu = 16{,}200\pm$.

V.

There can be no doubt that the controlling mechanisms at high and low temperatures are different. The harmony between the comparable averages for *Pterotrachea* and *Tiedemannia* is astonishing and very possibly not an accident. Yet within each list of high and low values, there are striking discrepancies. In the records, certain of these "increments" far above the average, and others as far below are attested by observations that can be interpreted, at present, in no other way. The precise meaning of these extreme variates and of certain intermediate irregularities remains problematical. Despite the remarkable agreements noted, certain temperatures, conceivably, may uncover in the heart rate one or more of a variety of processes which at other temperatures remain concealed. If this is a fact, then in certain states of equilibrium any one of these recessive reactions might be "exhumed" and fall momentarily into the position of control.

However, if we consider only the most frequent and closely concordant values, it seems safe to say that high temperatures disclose at least one controlling reaction whose characteristic lies between 7,000 and 8,000, while low temperatures uncover a second with its increment in the neighborhood of 22,000.

Are these or perhaps any of the other terminal values normal? From a purely physical standpoint this question is equivalent to asking whether a heart forced to disclose either both, or any, of the terminal increments, can return to the conditions that yield 11,200 or 16,200. Very likely if the animals were kept long enough at the extremes, the mechanism underlying the heart beat could be permanently altered. In these experiments the exposures were not sufficient to bring about such results. In every case a set of preliminary readings was taken at room temperature soon after the material had been brought into the laboratory. The animals were then cooled to the lower limit and when this was reached, usually after an hour, the first definitive observations were made. From this point on the temperature was increased step by step at regular intervals up to the maximum. In the intermediate zone, therefore, the performance of each individual occurred only after exposure to the lower thermal limit. The first reading at room temperature always coincides with the corresponding one made later on as part of the ascending series.

For the low values found at the upper limits, there are three checks. First, their general order of magnitude seems to rule out destructive effects such as irreversible coagulations or precipitations of proteins;

second, when the heart stops and after an intermission returns to rhythmical contraction with chambers properly synchronized, the results abruptly approach, or actually fall on the line characteristic for intermediate temperatures. This fact which at times makes the recognition of terminal increments difficult or impossible, is not without its compensations. Other possibilities granted, it suggests that during intermissions some essential material, exhausted, depleted, or "inactivated" by the great increase in heart rate, is restored to its original state or amount. Finally, in the case of *Pterotrachea* there is a special test. Individual 2 was run through the entire series of temperatures on 2 successive days. On January 26 in the range from 4–27°, the successive μ values were 20,600, 11,900, and 7,200; on January 27, from 4.6–26.8°, this animal gave respectively, 28,000, 11,900, and 6,900. Judging by their sense and absolute values and in view of the other considerations, these increments suggest no radical alterations in the underlying mechanism.

VI.

Within present limits of accuracy the intermediate increments of *Pterotrachea* and *Anodonta* on the one hand, and on the other of *Tiedemannia*, and *Limax* (Crozier and Stier), are identical. In sense and as averages the corresponding terminal characteristics for *Pterotrachea* and *Tiedemannia* are also the same. But homogeneity does not end with these indications. The fact that certain species exhibit increments of 16,000 ± for rhythmic movements under circumstances which elicit 11,000 ± from others, loses the qualities of an obstacle when we recall that in *Melanoplus* Crozier and Stier (1924–25, *b*) have experimentally changed the increment for abdominal rhythm from 16,500 to 11,300. For *Limax* the same authors (1924–25, *a*) find intermediate increments of 7,900 in December and 16,300 in March; while from Lang's results on *Helix*, they have deduced for the same temperatures μ values of 7,900 from data gathered in June, 11,300 from those of January, whereas February and March yielded 16,000. In all these instances the reaction in control at specific temperatures evidently differs as circumstances differ, yet the values at whatever temperature found, are always the same ones. What are the implications? Certainly in view of the taxonomic differences distinctive of the molluscs under consideration it would be hazardous to assume more than one catenary series of controlling reactions. Within the limits of viability, even this series, should further work lengthen it considerably, must be thought of as including at all times at least as many processes as there are trustworthy increments. Under certain conditions, most of which await precise definition, a particular region of the thermal scale may expose one reaction while under other circumstances the same temperatures may unearth quite another. Indeed, with the sole exception of artefacts or novelties introduced into the organism from without, a thermal field, biologically defined, can expose only normal constituents of the catenary system of control.

The logical consequences of this reasoning may appear ominous; and yet confusion and absurdity can be avoided even if thermal increments were to multiply equally for every biological process. After all, the total number of increments possible is probably restricted (Rice, 1923; Glaser, 1923; 1924–25, *b*). It is not difficult to imagine that circumstances might arise under which control could pass to chemical reactions which are not ordinarily in control. Our theory, at least, must be able to face the organism which discloses for all its measurable acts the same inventory of increments with differences only in serial order. In this somewhat academic event, analysis by means of temperature characteristics would be exhaustive because the series of catenary controls would contain as many items as there are types of reaction in the actual chemical system. If we could know where and how many times each type occurs, our diagnostic series and the actual system could be superimposed, point for point.[3]

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[3] The addition of every authentic increment is a step in the direction of the theoretical limit. For this reason the occurrence of two intermediate μ values in *Tiedemannia* seems important despite the absence of a convincing explanation. It is possible to assume differences in prelaboratory history, but whatever these may have been, the terminal increments remain unchanged. The lower value is not necessarily irreconcilable. Rare as it is, increments of this order associated with 11,000± and 16,000± have been reported in connection with respiratory processes and those that might be limited by the rates of gaseous exchange. Arrhenius (*loc. cit.*, p. 55) lists as a mean value for cell division in certain eggs, 14,100 and attaches to "respiration in plants," 14,800. According to Crozier (1924–25, *b*), Krogh's data on respiration in dogs under curare yield μ = 13,780.

VII.

The low increments characteristic of high temperatures are well supported in the literature. Somewhere in the catenary chain underlying respiratory phenomena in general and phenomena controlled by respiration, is a reaction whose increment is between 7,000 and 8,000. In certain instances, such as translation in *Paramecium* (Glaser, 1924–25, *a*) and the heart rates of *Pterotrachea* and *Tiedemannia*, it requires a fairly high temperature to expose this reaction whereas in *Melanoplus*, as Crozier and Stier (1924–25, *b*) show, it may control in normal or decapitated individuals over the entire range. To these writers (*loc. cit.*) also, we owe an interesting suggestion. Between 15° and 38° Bodine's measurements on CO_2 production in *Melanoplus* yield $\mu = 7{,}710 \pm 700$, whereas the data of Batelli and Stern (Crozier and Stier), perhaps doubtful on account of the high temperatures employed (between 30° and 40°), yield an increment of 8,000 ±. Crozier and Stier plainly imply a relation between this value and the excretion of CO_2.

For the increment of 22,000 ± at low temperatures, a provisional assignment is perhaps also possible. It is found frequently associated with 11,000 ± and 16,000 ± (Crozier, 1924–25, *b*). Under laboratory conditions the blood of *Panulirus* undergoes a rapid loss of

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With the exception of cleavage, all these processes under different circumstances have also given $\mu = 16{,}000\pm$ or $11{,}000\pm$, or both. It is not impossible then that increments of 14,000± are normally recessive in respiratory phenomena but for one reason or another, not apt to emerge.

The association of this value with curare is suggestive. *Tiedemannia*, as observed, moves its "wings" rhythmically. If the individual happens to be a small one it is often necessary to await those recurrent periods when the individual is completely quiescent. Just prior to such an interval the movements become slower and the excursions more prolonged—symptoms found during the rhythmical phase of reflexes approaching fatigue (Bayliss, 1924; p. 501). If we suppose that *Tiedemannia* comes to rest for the same or comparable reasons, an explanation for the value 14,500± might be sought and perhaps found in the element common to the effects of curare and of certain fatigue substances, curare eliminating the final motor neurone by affecting a "receptive" material in the muscle and "fatigue" deleting the same neurone by blockade at the proximal synapse (*cf.* Bayliss, *loc. cit.*, p. 399 *et seq.*). Until the situation is clarified, however, we must withhold from the increment of 14,500± the consideration given to the others.

glucose (Morgulis; *cf.* Crozier, 1924–25, *b*). In starving crayfish, Brunow's figures on the utilization of O_2 and the production of CO_2 yield $\mu = 22{,}000$ (Crozier, *loc. cit.*). It is easy to imagine that low temperatures might reduce, possibly reduce differentially, either the solubilities or the rates upon which a normal supply of glucose depends. If this is true, such temperatures would bring about changes in equilibrium closely comparable and perhaps identical with those characteristic of inanition. The temptation to attach 22,000 to hydrolytic reactions in which a substrate is prepared for oxidation is recognized by Crozier (*loc. cit.*).

VIII.

We can now attempt to picture the controlling mechanism for heart rate in molluscs, provisionally, as a catenary series of at least four reactions. Of these, the one with the smallest increment controls at high temperatures and the one with the largest value at low. Between these extremes control may fall to either of the remaining two, and these, as in *Melanoplus*, should be capable of being artificially substituted, one for the other.

Although we are not dealing exclusively with a muscle, it is perhaps not unreasonable to consider glycogen as a source of energy. Assuming an adequate original supply, O, a mechanism modelled on Meyerhof's (1924) conception of carbohydrate metabolism appears capable of accounting for the observed velocity controls. We can attach the increment of 22,000 ±, directly or *via* some catalyst, to a mobilization hydrolysis, $O \rightarrow A$. In a similar manner 11,000 ± may be assigned to an "oxidation," $A \rightarrow B$, very likely catalyzed by OH'; while 16,000 ± may be taken to characterize a reaction $B \rightarrow E$ which liberates the energy and is catalyzed otherwise. The increment 7,000 ± would then fall to the resynthesis $E \rightarrow O$ (lactic acid → glycogen?), and on occasion also to any other pertinent reaction involving the production of CO_2 and important in maintaining the original source of supply.

While the interpretations on which this particular scheme is based should involve no irrevocable commitments, it has, nevertheless, certain advantages. For one thing, its form projects into the substrate the cyclical nature of rhythm, and, resting on the apparently solid foundation laid by Meyerhof (*loc. cit.*) brings under one rubric phenomena superficially diverse. As a formulation we are obviously dealing merely with the harmonious enlargement of an idea suggested as applicable to linear translation in *Paramecium* (Glaser, 1924–25, *a*), where the thermal field so far has exposed only two values: 16,000 from 6–15° and 8,000 for the higher ranges. The interpretation of these values remains unchanged. As Crozier and his collaborators (1924–25) have shown, increments of 11,000 ± and 16,000 ± are characteristic of processes involving O_2 consumption and the production of CO_2.

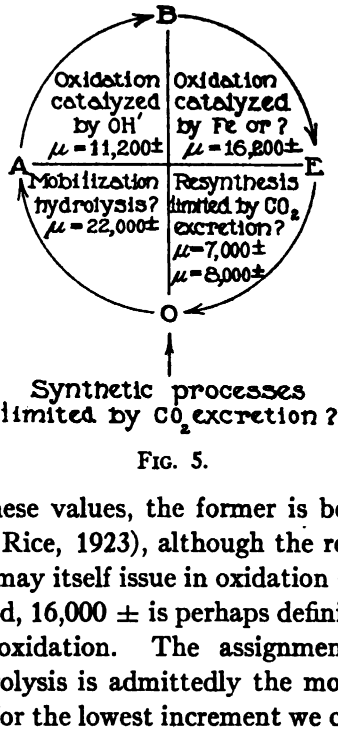
B
A
E
O
Oxidation catalyzed by OH′ $\mu = 11{,}200\pm$
Oxidation catalyzed by Fe or ? $\mu = 16{,}200\pm$
Mobilization hydrolysis? $\mu = 22{,}000\pm$
Resynthesis limited by CO_2 excretion? $\mu = 7{,}000\pm$ $\mu = 8{,}000\pm$
Synthetic processes limited by CO_2 excretion ?

FIG. 5.

Of these values, the former is believed to reveal the activity of OH′ (*cf.* Rice, 1923), although the reaction in which this intervention occurs may itself issue in oxidation (Oppenheimer, 1925, page 225); the second, 16,000 ± is perhaps definitely to be associated with a catalyzed oxidation. The assignment of 22,000 ± to a mobilization hydrolysis is admittedly the most speculative of all. On the other hand, for the lowest increment we can appeal to at least one other synthetic process. In the data of Miss Leitch (Crozier, 1924–25, *b*) μ below 15° is 8,170 for growth in the radicle of *Pisum*. If in the synthesis $E \rightarrow O$ the excretion of CO_2 should be a limiting factor, it is interesting to recall (Meyerhof) that the reaction which transforms lactic acid into glycogen also sets free six molecules of CO_2. Finally, the place assigned to the lowest increment makes it conceptually easy to link the immediate cycle underlying the heart rate with the rest of the organism because the synthesis of O is not restricted to movements from E, but in the model may result, as it does in nature, from reactions spatially distinct yet with the same control.

SUMMARY.

1. For the heart rate in *Pterotrachea coronata*, intermediate temperatures disclose a thermal increment of 11,200 ±. This value is identical with the one reported by Crozier and Stier for the lamellibranch, *Anodonta*. In the pteropod, *Tiedemannia neapolitana* the same temperatures typically reveal in the heart rate a μ value of 16,200 ±. This agrees quantitatively with 16,300 found by Crozier and Stier for the heart of the slug, *Limax maximus*.

2. At high temperatures the average value of μ for *Pterotrachea* is 7,300: for *Tiedemannia*, 7,400. The corresponding averages at the lower limits are 22,000 and 23,000.

3. The great variability found near the edges of the temperature field are explicable in two ways. During intermissions characteristic of high temperatures and occurring also at low, we can assume a restorative process; while at both the upper and lower limits we may, in addition, find that reactions assume control which under ordinary circumstances never do so. Special evidence indicates that the highest temperatures employed, 27°C., and the lowest, 4°C., caused no irreversible changes in mechanism.

4. The theoretical analysis of the experimental facts makes use of Meyerhof's conception of carbohydrate metabolism and projects the cyclical nature of rhythm into the substrate of control. Assuming as a source of energy an original supply of material O, the value of 22,000 ± is assigned provisionally to a mobilization hydrolysis while 11,200 ± and 16,000 ± are attached to oxidative reactions influenced respectively by OH' and possibly Fe, or some other catalyst. The lowest value, 7,300 ± is assumed to indicate a synthetic process (lactic acid → glycogen?), possibly limited by CO_2 excretion. In the present state of our knowledge, this distribution and interpretation seems to account reasonably for the experimental facts, but until we know more about the neurogenic controls, is entitled to rank only as an hypothesis.

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